Characterisation of the Superior Colliculus in a Rat Model of Attention Deficit/Hyperactivity Disorder

Thesis

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Characterisation of the Superior Colliculus in a Rat Model of Attention Deficit / Hyperactivity Disorder

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BSc. (Hons) Neuroscience

A thesis submitted for degree of Doctorate of Philosophy in the Discipline of Neuroscience

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Thomas Gray (1716–1771): "Not all that tempts your wand'ring eyes

and heedless hearts, is lawful prize; Nor all, that glisters, gold."

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ABBREVIATIONS

5-CSRTT	5-choice serial reaction time test
5-HT	Serotonin
6-OHDA	6-hydroxydopamine
ACB	Nucleus accumbens
ADHD	Attention Deficit/ Hyperactivity Disorder
AMPH	Amphetamine
ANOVA	Analysis of variance
AP	Anterior-posterior
Aq	Cerebral aqueduct
asf	area sampling fraction
ATX	Atomoxetine
BOLD	Blood-oxygenation-level-dependent
BRU	Biomedical Resource Unit
BSA	Bovine serum albumin
CE	Coefficient of error
CNS	Central nervous system
CPT	Continuous performance task
DA	Dopamine
DAB	Diaminobenzidine
DAT	Dopamine transporter
DAT-KO	Dopamine transporter knockout
DLPFC	Dorsal lateral prefrontal cortex
DpG	Deep grey
DpW	Deep white
DPX	Distyrene Plasticizer Xylene
DR	Delayed reinforcement
DSM 5	Diagnostic and Statistical Manual Edition 5
EEG	Electroencephalography
EXT	Extinction
FEF	Frontal eye fields
FI	Fixed Interval
FLUX	Fluoxetine
GABA	Gamma-aminobutyric acid
GH	Genetically hypertensive rat
HL	Hooded Lister rat
hsf	height sampling fraction
i.v.	intravenously
IC	Inferior colliculus
InG	Intermediate grey
InWh	Intermediate white
LC	Locus coeruleus
LGN	Lateral geniculate nucleus
LIP	Lateral intraparietal cortex
LS	Lateral supersylvian

MAOA	Monoamine oxidase A
MGS	Memory guided saccades
ML	Medial-lateral
MPH	Methylphenidate
MST	Medial superior temporal area
MT	Medial temporal area
NA	Noradrenalin
NET	Noradrenalin transporter
NMDA	N-methy-D-aspartate
Op	Opticum
PAG	Periaqueductal grey
PB	Phosphate buffer
PBS	Phosphate buffer saline
PFC	Prefrontal cortex
PSTH	Peri-stimulus time histograms
PULd	Dorsal pulvinar
PULi	Inferior pulvinar
s.c.	subcutaneously
SC	Superior Colliculus
SD	Sprague Dawley
SERT	Serotonin transporter
SNAP-25	Synaptosomal-associated protein 25
SHR	Spontaneously Hypertensive Rat
SNr	Substantia nigra pars reticulata
SNRI	Selective noradrenalin reuptake inhibitor
SPECT	Single photon emission computed tomography
SRS	systematically random sampled
ssf	section sampling fraction
SSRI	Selective serotonin reuptake inhibitor
SSRT	Stop-signal reaction time
SuG	Superficial grey
TS	Tris buffered saline
V1	Visual cortex
VGS	Visually guided saccades
VTA	Ventral tegmental area
WIS	Wistar
WKY	Wistar-Kyoto
Zo	Zonal layer

ABSTRACT

Attention-deficit/hyperactivity disorder (ADHD) is a neurobehavioural disorder of childhood onset. Core symptoms include hyperactivity, impulsivity and inattention. Despite high prevalence and effective pharmacological treatment, the pathophysiology is poorly understood. Present theories of the etiology of ADHD suggest a crucial influence of dopamine. To date, little investigation has focussed on structures upstream of dopamine neurons which could cause these abnormalities. The midbrain superior colliculus (SC) is conserved across species and plays a role in saccade generation, visual saliency and attention. Evidence suggests that the SC could be dysfunctional in ADHD, and may explain core symptoms of ADHD, providing a site for action of therapeutic treatments. The spontaneous hypertensive rat (SHR), an animal model for ADHD has shown face validity, construct validity and predictive validity, and is the most commonly used animal model of ADHD. Understanding the etiology of the ADHD-like behaviours in the SHR is important in improving our understanding of the etiology of ADHD itself. This thesis presents work that demonstrates that the SHR responds to visual and auditory stimuli in a different way behaviourally and physiologically compared to two control strains, with these differences likely to be mediated by alterations within and upstream of the SC, respectively, resulting in altered saliency of sensory stimuli. These results are compatible with the two unifying theories of ADHD, but suggest that the dysregulation of dopamine is secondary to changes upstream in the SC and structures projecting to it. The findings of slower onset latency in the SHR are also in line with ADHD and the ADHD-like behaviours seen in the SHR and support ADHD being a development disorder. ADHD treatments such as amphetamine and fluoxetine may have a mechanism of action within the SC, and therefore normalise the exaggerated response, yet the results from the current experiment on drug effects are inconclusive.

1. INTRODUCTION

1.1.ATTENTION-DEFICIT/HYPERACTIVITY DISORDER

Prevalence and consequences of ADHD

Attention-Deficit/Hyperactivity Disorder (ADHD) is the most common neurobehavioural developmental disorder. The prevalence of ADHD is estimated to be 3–9% in school-aged children and young people in the UK (National Institute for Health and Care Excellence, 2008). In 30–60% of individuals with ADHD, the disorder persists into adulthood (Cantwell, 1996; Ulloa et al., 2005), with a National Comorbidity Survey Replication (2006), estimating 4.4% of adults between ages 18 and 44 experience some symptoms and disabilities from ADHD in the United States.

As well as being a very prevalent disorder, ADHD is extremely debilitating. Children diagnosed with ADHD are at a higher risk of having learning, behavioural, and emotional problems throughout childhood, adolescence and adulthood (Barkley et al., 1991). Indeed, of the children diagnosed with ADHD, 50-70% have problems related to social adjustment and functioning (Cantwell, 1996). They have been shown to have difficulty with social interactions both with peers (Flicek, 1992) and family members (Barkley et al., 1991). Children with ADHD will also often experience other psychiatric problems as adolescents and young adults (Cantwell, 1996). Several comorbid disorders can be found in children and adolescents with ADHD, including oppositional defiant disorder and conduct disorder (50%), anxiety disorders (25–35%), mood disorders (15%) and learning disabilities (25%; Biederman et al., 1991). The latter includes reading disorder, dyslexia, dyscalculia and problems with writing. It is particularly common in boys with ADHD, with 66% of boys with ADHD being diagnosed with another behavioural disorder (Biederman et al., 2002a). The estimate of 25% for learning disabilities may even be conservative and comorbid learning disabilities range from 7% to 92%, depending on the definitions used (DuPaul and

Stoner, 1994). Compared to those without ADHD, adults diagnosed with ADHD as children spend fewer years at school, achieve lower overall occupational status, and have an increased likelihood of being diagnosed with psychiatric problems such as antisocial personality disorder and a higher risk of drug abuse (Mannuzza et al., 1998).

Diagnosing of ADHD and core symptoms

ADHD is diagnosed on the basis of persistent and developmentally-inappropriate levels of inattention and/or hyperactivity-impulsivity. It is important to note that children without ADHD are impulsive, hyperactive and inattentive to some degree and may exhibit such behaviour at different times and at different stages of development. The diagnosis of ADHD is thus based not only on the presence of these symptoms but also on their expression to an abnormally persistent and developmentally inappropriate extent.

Two main tools are available for diagnostics: the Diagnostic and Statistical Manual 5 (DSM 5) from the American Psychiatric Association and the International Classification of Diseases, 10th revision (ICD-10) from the Word Health Organisation. Both tools can be used by mental health professionals to help diagnose ADHD. DSM 5 replaced the previous version, DSM-IV-TR in May 2013, although it is worth noting that the diagnostic criteria have not changed significantly in terms of symptom type between the two versions. According to DSM 5, for ADHD to be diagnosed symptoms must have been present for a minimum of six months. The number of symptoms present differs according the age of the individual. For children up to the age of 16, at least 6 symptoms must be present whilst for adolescents 17 or older and adults, at least 5 symptoms are needed for a diagnosis. These symptoms can be divided into inattention or hyperactivity/impulsivity, as shown in Table 1.1.

Inattention	Hyperactivity/ Impulsivity
Fails to give close attention to details, or makes careless mistakes with work.	Often fidgets with/or taps hands or feet, or squirms in seat
Often has trouble holding attention in tasks	Often leaves seat in situations when it is not allowed
Often does not seem to listen when spoken to directly	Often unable to take part in leisure activities quietly
Often does not follow through on instructions, and fails to finish schoolwork, chores	Is often 'on the go' as if 'driven by a motor'
Often has trouble organising tasks and activities	Often talks excessively
Often avoids doing tasks that require mental effort over a long period of time	Often blurts out answers before appropriate
Often loses things necessary for tasks and activities	Often has trouble waiting his/her turn
Is often easily distracted	Often interrupts or intrudes on others
If often forgetful in daily activities	

Table 1.1: An outline of the diagnostic criteria for ADHD in DSM 5 (Diagnostic and Statistical Manual 5, American Psychiatric Association, 2013).

Based on symptoms presented in the table, which are described in detail in Section 1.1.1, the DSM 5 differentiates between three clinical subtypes or presentations based on the presence of different core symptoms:

1. Predominantly inattentive presentation - Enough symptoms of inattention, but not hyperactivity/impulsivity, were present for the past six months.
2. Predominantly hyperactive/impulsive presentation: Enough symptoms of hyperactivity/impulsivity, but not inattention, were present for the past six months
3. Combined presentation: Enough symptoms of both inattention and hyperactivity/impulsivity were present for the last six months.

It is noteworthy that symptoms can change over time and consequently so can the presentation diagnosed. Interestingly, the inattentive subtype of ADHD is most prevalent in female suffers (Taylor et al., 1998). Girls with ADHD have been shown to be more anxious and with less disruption within their behaviour and lower rates of hyperactivity (Novik et

al., 2006; Gaub and Carlson, 1997), yet no gender differences have been seen in impulsiveness, peer functioning and academic performance (Gaub and Carlson, 1997).

The ICD uses a different classification for ADHD in comparison to the DSM 5 and outlines a stricter set of requirements for diagnosis. The same types of symptom are defined; inattention, hyperactivity and impulsivity in the context of hyperkinetic disorders of childhood, but all the symptoms must be present. In effect, this means that only the 'combined-presentation' ADHD found by DSM 5 will meet the requirements for an ADHD diagnosis using ICD 10. Furthermore, the symptoms must be verified as present by two different sources in at least two environments, for example, at home and at school. A singular source, for example, parental reports of both schooling and home behaviour would not be sufficient for diagnosis. Within the ICD guidelines, comorbid psychiatric disorders such as anxiety will not produce a comorbid diagnosis of hyperkinetic disorder unless it is clear that hyperkinetic disorder is additional to the other disorder. Hyperkinetic disorder therefore describes a group that forms a severe sub-group of the DSM 5 combined subtype of ADHD. Adults may also fail to receive appropriate diagnosis of ADHD when using ICD 10 as the criteria focuses on childhood problems, and do not take into account the developmental changes seen in ADHD and accounted for in the DSM 5.

1.1.1. CORE SYMPTOMS

Inattention

A deficit of sustained attention and increased distractibility is evident in children (Conners et al., 1996; Oades, 1987; Wigal et al., 2005; Faraone et al., 2007; Brown and Cooke, 1994) and adults with ADHD (Adler and Chua, 2002). Evidence for this has come from a number of different experimental paradigms. One such paradigm used to assess sustained attention is the computerised Continuous Performance Test (CPT). The test primarily assesses

attention and impulse control (Conners, 1985) and involves the presentation of target and non-target stimuli. Briefly, participants are required to respond to a stimulus on a computer screen by pressing a space bar for every stimulus except for one specific stimulus (the target stimuli). Optimal performance on the CPT is achieved by responding quickly and not making mistakes throughout the duration of the task (Pearson et al., 2004). Wang et al. (2011), demonstrated that children with ADHD have increased errors of commission (incorrect responses - pressing when not appropriate) and omission (not pressing when appropriate), which are assumed to reflect impulsivity and inattention, respectively.

In addition to this direct measure of attention, some researchers have speculated attentional deficits exist from learning paradigms. In particular, researchers have examined extinction patterns when discontinuing reinforcement for a previously reinforced behaviour. Proportional analysis of data from children with and without ADHD performing the same basic extinction (EXT) task, showed differences in the underlying pattern of behaviour and response rates (Iaboni et al., 1997; Sagvolden et al., 1998). Research carried out on adults with ADHD during an EXT task found similar results (Lee and Zentall, 2006). In the case of Sagvolden et al. (1998), the apparatus was of a clown's face, where the clown's eyes (the signal) were lit during the 30-s fixed interval (FI) schedule component and turned off during the 120-s EXT component. During FI, the first press on the nose (the lever) during the 30-s interval was reinforced by the delivery of a trinket or a coin in the clown's mouth. By contrast, reinforcers were not delivered during the EXT component. The children with ADHD initially stopped the reinforced behaviour response when the signal, the clown's eyes, was turned off and then resumed responding some time thereafter as if the signal had been turned on again. By contrast, children without ADHD ceased responding permanently during extinction. The authors suggest that in children with ADHD, behaviour during the extinction component may well be related to a sustained-attention deficit, as the children did not discontinue the previously reinforced behaviour. It is important to

recognise that this is not direct a measure of sustained attention and is more an assessment of the degree of learned reinforced behaviour, which may reflect deficits in sustained attention but can also be linked to motivational processing, with the children with ADHD showing increased reward-seeking behaviour (Sonuga-Barke et al., 1992).

It is important to note that the presence of these symptoms does not necessarily mean an inability to attend selectively, as is sometimes assumed. On the contrary, children with ADHD are able to focus attention during tasks that involve a high rate of immediate reinforcement. Furthermore, apparent attention deficits can be reduced or eliminated when playing video games or performing tasks for large amounts of money immediately on completion (Barkley, 1990). However, when the intensity of reinforcement is decreased, behaviour becomes readily distinguishable from children without ADHD (Barkley et al., 1990). Such observations may suggest that ADHD is not a primary deficit in attention mechanisms, but rather more a problem of the manner in which behaviour is regulated by its effects or consequences (Draeger et al., 1986; Haenlein and Caul, 1987). Alternatively, there may be non-contingent effects of reinforcers that provide additional stimulation. Inattentive behaviour is poorly operationalised, and judgments about inattentiveness are indirect conclusions from observed behaviour. Thus, behavioural alterations causing poor test scores on measures of attention may be misinterpreted (McGaughy, 1996).

Hyperactivity

Studies investigating hyperactivity in children with ADHD commonly use actometers (acceleration-sensitive devices) capable of providing an overall measure of activity to provide an objective measure of activity level. These studies show that children with ADHD are more active overall than children without ADHD in natural situations such as within the classroom (Hoeger and Mace, 2006). Objective activity measures using actometers have also shown, however, that hyperactivity is sensitive to context. Hyperactivity is modulated

by situational variables and may be indistinguishable from healthy levels of activity when there is sufficient stimulation as noted by Felicetti and Julliard (2000) observing hyperactivity during a dental visit. One way in which this sufficient stimulation may materialise is through novelty. Children with ADHD only display locomotor hyperactivity in a familiar environment such as a classroom, but not in a novel environment in the case of the dental visit (Felicetti and Julliard, 2000). This influence of degree of novelty of a situation on activity levels argues against a constant, over-activity in ADHD. Furthermore, these findings suggest that ADHD is not primarily a deficit in motor mechanisms but rather a problem of the manner in which behaviour is regulated by its effects or consequences (Draeger et al., 1986; Haenlein and Caul, 1987). It is noteworthy that, even though ADHD is still present in a high percentage of adult diagnosed in childhood, the hyperactive symptoms often diminish (Adler and Chua, 2002) and therefore the symptom profile of an individual with ADHD may change over time.

Impulsivity

Increased impulsivity is the third core behavioural feature associated with ADHD but exactly how impulsivity is defined is the subject of debate. Some researchers focus on a lack of inhibitory control as the core feature of impulsivity in ADHD (Quay, 1997), whilst others emphasise a greater intolerance to delay of reinforcement (Logue, 1988). These two aspects may be independent, but they are also interrelated, in that a greater preference for immediate gratification may aggravate difficulty withholding a response. The concepts of intolerance to delay of reinforcement and lack of inhibitory control are both important in ADHD and will be considered in more detail below (see Section 1.1.6). However, at this stage it is important to note that both of these elements of impulsivity are incorporated into DSM 5 with reference to a preference for immediate reinforcement ('difficulty waiting') and difficulty withholding responses ('blurts out'). Furthermore, there is experimental evidence for alterations to both elements of impulsivity. For example, children with ADHD have been

shown to prefer small immediate reinforcers over larger yet delayed reinforcers, suggesting that the children with ADHD are more concerned with reducing the overall delay of reinforcement than maximising the reward amount (Antrop et al., 2006; Sonuga-Barke et al., 1992, Pardey et al., 2009; Marco et al., 2009). Marco et al. (2009) assessed the contribution of impulsive drive for immediate rewards and delay aversion to whether children chose a small immediate reinforcer over larger yet delayed reinforcer. Individuals with ADHD chose small immediate reinforcers more than those without ADHD irrespective of whether or not the small immediate reinforcer led to a greater overall trial delay. It was also noted that children with ADHD who preferred the small immediate reinforcers were younger and had a lower IQ. From a familial perspective it was found that children with ADHD who preferred the small immediate reinforcers were more likely to have siblings with a similar preference of reinforcement. A dual component model in which both impulsive drive for immediate rewards and delay aversion contribute to the choice of reinforcer has been suggested (Marco et al., 2009), and these behaviours may be caused by altered reinforcement processes (Sonuga-Barke et al., 1992).

A paradigm developed to directly measure and clarify the mechanisms underlying the inhibition of an ongoing motor response is the stop-signal paradigm, the individual is asked to respond quickly and accurately to a discrimination task. The participants are prompted to inhibit their response if a stop signal is shown shortly after the primary task stimulus. A stop-signal reaction time (SSRT) estimating the latency of the internally generated inhibitory process can be produced by manually varying the interval between the onset of the go stimulus and the onset of the stop signal (termed the stop-signal delay). Poorer discrimination accuracy, as shown through an increase in choice errors in pressing the button during the stop signal, suggests an impulsive response (Quay, 1997). Previous studies have consistently found poorer inhibitory performance in children with ADHD relative to controls, with longer SSRTs and a high percentage of failed inhibitions (Dimoska

et al., 2003). Dimoska et al. (2003) suggested impairment in the sensory registration and identification of the stop signal in ADHD occurring at the early stages of inhibitory processing.

1.1.2. TREATMENT

Pharmacotherapy

Currently, two major classes of drugs are used in the treatment of ADHD: psychostimulants and non-psychostimulants. The psychostimulants used are amphetamine (AMPH), usually supplied as 3:1 mixture of d- and l- isomers, for example, under the trade name of Adderall, or methylphenidate (MPH), usually supplied as dl-threomethylphenidate, for example, under the trade name of Ritalin, or as a slow release preparation, Concerta. The non-psychostimulant treatments include selective noradrenalin reuptake inhibitors (SNRI) including atomoxetine (ATX), available under the trade name of Strattera, and selective serotonin reuptake inhibitors (SSRI) such as fluoxetine (FLUX), which is available under a variety of trade names including Prozac.

Pharmacotherapy with psychostimulants dates back to 1937 when Bradley (1937) discovered that amphetamines ameliorate disruptive behaviour in children. The psychostimulants affect central catecholaminergic neurotransmission causing an accumulation of synaptic levels of the monoamines dopamine, noradrenalin (Azzaro and Rutledge, 1973; Easton et al., 2007b) and, at elevated doses, serotonin (Holmes and Rutledge, 1976; Kuczenski and Segal, 1989). AMPH and MPH improve sustained attention, and suppress distractibility in individuals without ADHD (Silber et al., 2006; Halliday et al., 1990; Agmo et al., 1997), as well as in individuals with ADHD (Oades, 1987; Brown and Cooke, 1994; Spencer et al., 2001; Wigal et al., 2005; Faraone et al., 2007). At the low doses used in the treatment of ADHD, these drugs are devoid of locomotor-activating effects, and instead reduce movement and impulsivity and increase cognitive function, including

sustained attention and working memory (Solanto 1998). However, at moderate and high doses, they can cause behavioural sensitisation leading to impaired cognition and exert pronounced reinforcing and locomotor-activating actions. These are all factors potentially leading to substance abuse (McGaughy and Sarter, 1995; Rebec and Bashore, 1984; Segal, 1975). Importantly, these actions occur in both individuals with and without ADHD (Elliott et al., 1997; Mehta et al., 2001; Rapoport et al., 1980; Rapoport and Inoff-Germain, 2002; Solanto, 1998; Vaidya et al., 1998; Wilens et al., 2004). The differential effect of low and high doses is thought to be related to the effects on the prefrontal cortex (PFC). Berridge et al. (2006) found that in contrast to the widespread activation of catecholamine efflux within the brain observed with higher doses of MPH and other psychostimulants (Kuczenski and Segal, 1994), low-dose MPH selectively activates noradrenalin (NA) and dopamine (DA) neurotransmission within the PFC which may contribute to the motor-calming actions of low-dose stimulants. Alternatively, it has been proposed that ADHD with and without hyperactivity involves differential participation of the PFC and basal ganglia, respectively (Diamond 2002). Thus, the preferential targeting of PFC catecholamines by low-dose MPH may be of particular relevance for the treatment of ADHD without hyperactivity.

Presently, psychostimulants are the treatment of choice for ADHD. However, despite their recognised efficacy and safety, their chronic use during adolescence has been criticised. Adolescent exposure to methylphenidate has been shown to trigger lasting changes in rats, such as long-term modulation of self-control abilities, reduced sensitivity to natural and drug reward, as well as augmented stress-induced emotionality (Macro et al., 2011). In addition, due to a variety of crucial factors, for example a total or partial lack of response to treatment, or intolerance to side effects (loss of appetite, sleep alterations, mood swings), 10-30% of individuals with ADHD discontinue psychostimulant treatment (Barkley 1977; Elia 1991; Wilens, 2006). Therefore, alternative treatments for ADHD are needed

(Banaschewski et al., 2004) and non-psychostimulant treatments are being increasingly used by a significant proportion of individuals with ADHD.

The non-psychostimulant drug atomoxetine is the only non-psychostimulant approved by the Food and Drug Administration for the treatment of ADHD. It has been found to be an effective treatment for the disorder in several randomised, double-blind, placebo-controlled, clinical trials (Spencer et al., 1998; Biederman et al., 2002b; Michelson et al., 2002; Spencer et al., 2002). It is generally well tolerated in children and adolescents, with mild adverse effects. It also lacks the abuse potential of psychostimulants and is often a useful treatment for those who do not tolerate psychostimulants (Virani, 2005; Dittman et al., 2013). Dittman et al. (2013) in a 9 week double blind study found that 66% of children who did not respond adequately to amphetamine did respond to atomoxetine. However, there have been concerns regarding increased suicidality in sufferers using atomoxetine, and unlike the rapid response seen with psychostimulants, some patients require 3 to 4 weeks of atomoxetine therapy before improvements are seen (Virani, 2005).

Atomoxetine is a selective noradrenalin reuptake inhibitor with relatively low affinity for serotonin and dopamine uptake sites (Bolden-Watson and Richelson, 1993). However, despite the selectivity of atomoxetine, it does actually increase dopamine and noradrenalin in prefrontal cortex at clinically relevant doses because the noradrenalin transporter plays an important role in clearance of dopamine in this region (Bymaster et al., 2002; Swanson et al., 2006). Furthermore, atomoxetine has been found to bind with the serotonin transporter (SERT) at clinically-relevant doses displaying dose-dependent occupancy on both noradrenalin transporter (NET >90%) and SERT (85%) (Ding et al., 2014). Similarly, amphetamine, but not methylphenidate, significantly increased the extracellular levels of serotonin (5-HT) in the nucleus accumbens, whilst methylphenidate-induced locomotor activation was found to be mediated by 5-HT_{1B} receptors in the rat (Borycz et al., 2008).

The effects of both psychostimulants and atomoxetine on serotonin levels, and a potential role for this neurotransmitter in pharmacotherapy for ADHD are also supported by evidence that fluoxetine, a serotonin selective reuptake inhibitor, has therapeutic efficacy in ADHD (Barrickman et al., 1991). In a 6-12-week open-label study of fluoxetine therapy for children with ADHD, fluoxetine was associated with a significant decrease in inattention/hyperactivity and aggression/defiant symptoms. Forty seven percent of participants much or very much improved without observed adverse effects (Quintana et al., 2007).

Non-pharmacological treatment

For the sake of completeness it should be recognised that non-pharmacological treatments are also available. These include cognitive/behaviour modification therapy, educational interventions, EEG neurofeedback and dietary changes. Typical behaviour modification therapies include positive attention for appropriate behaviours, and withdrawal, extinction, or punishment for non-compliance. The limitations of behaviour therapy include the need for continued intervention, the complexity of the therapy, dependence on parent–teacher cooperation, and high cost (Barabasz and Barabasz, 2000; Grantham, 1999). Kendall et al. (1980) developed a self-control program called 'Stop and Think'. This addressed hyperactivity and impulsivity by training children to improve their concentration and reflection skills. The Stop and Think technique employed problem-solving, self-instruction, modelling, role-playing, and reinforcement systems. Miranda and Presentacion (2000) combined Stop and Think with anger control procedures on ADHD children both with and without hyperactive-aggressiveness. Their anger control procedures consisted of identifying the physiological, cognitive, and affective cues of anger, coupled with relaxation techniques. The results of the combined therapy produced long-term positive effects on internalisation problems and anti-social behaviour. Improvements using the combined

approach were better than the use of Stop and Think alone. However, their approach did not produce significant changes in either school performance or social adjustment.

In the Elton report (HMSO, 1989) it is stated that 'a teacher's general competence has a strong influence on his or her pupils' behaviour'. Educational intervention includes improving teachers' knowledge of ADHD alongside providing advice on how to work with children who might have ADHD and may improve outcomes. The effectiveness of giving advice to teachers in addition to a parent-training programme was large in reducing children's ADHD core symptoms as rated by both parents and teachers (Corkum et al., 2005). In summary, there is some evidence that advice to teachers as an added intervention to a parent-training programme is effective in reducing children's ADHD core symptoms. Meta-analysis determined that educational interventions resulted in higher cognitive outcomes, while pharmacological interventions resulted in higher behaviour outcomes (Purdie et al., 2002).

The basis of EEG neurofeedback is that individuals could learn to control inappropriate impulses without drugs or extensive counselling. Among the more promising alternatives to drug treatment, neurofeedback uses advanced electronics and computerised mathematical computations to convert EEG patterns into images and sounds on a video display. Based on the principle of operant conditioning, children with ADHD learn to control and normalise the areas of the brain that are associated with a dysfunction in the EEG pattern. A direct comparison of medication versus neurofeedback showed similar therapeutic results, as well as significant improvements in intellectual performance with neurofeedback, without any negative side-effects (Sterman, 2000). In addition, studies showed that EEG neurofeedback resulted in significant and sustained physiological changes in both animals and humans, even during sleep (Barabasz and Barabasz, 2000). A limitation of

neurofeedback is that a large number of treatment sessions (80 sessions over six to eight months) are required to produce lasting effects.

The list of proposed dietary treatments includes sugar-restricted, additive- and salicylate-free diets (Feingold diet), and similarly, a study also links ADHD in adolescents with a "Western-style" dietary pattern, high in fat, refined sugars, and sodium and low in fibre, folate, and omega-3 fatty acids (Howard et al., 2011). The Feingold diet includes the avoidance of foods such as apples, grapes, luncheon meats, sausage, hot dogs, and cold drinks containing artificial flavours and colouring agents. It was popularised in the 1970s and accepted by many parents. Systematic reviews, however, showed that the diet was not effective and concluded that food additives do not cause ADHD (Conners et al., 1980). An increase in hyperactivity is often reported by parents after a child with ADHD eats an excessive amount of sweets or fizzy drinks but the majority of controlled studies failed to demonstrate a significant adverse effect of sucrose or aspartame (Wolraich et al., 1994). Children are more vulnerable to reactive hyperglycaemia, and this has been suggested to induce sugar induced cognitive impairment and inattention (Shaywitz et al., 1994). Therefore the avoidance of rapidly absorbed sucrose-containing foods in young children may prevent diet-related exacerbations of ADHD.

A zinc deficiency has also been suggested to exacerbate ADHD symptoms, with individuals with ADHD reportedly having low zinc levels in hair and urine (Arnold et al., 2005). Low serum zinc levels correlated with parent/teacher-rated inattention yet ratings on hyperactivity and impulsivity were not affected (Arnold et al., 2005), and zinc monotherapy reportedly improved ADHD symptoms (Bilici et al., 2004). Zinc regulates dopamine metabolism as well as the metabolism of other neurotransmitters and fatty acids, in areas of the brain linked to ADHD.

Although all behavioural approaches seem to provide a long-term benefit, drug therapy was superior to behavioural therapy in managing ADHD symptoms/manifestations and a combination of the two did not have an additive effect (MTA Cooperative Group, 1999). Behavioural therapy was useful, but the effect was not as robust as for drug therapy. However, in other studies, behavioural therapy has been shown to be equally efficacious as psychostimulants administered at low doses (MTA Cooperative Group, 1999). Therefore, it may be useful for ADHD patients with mild symptoms and minimal impairment or when parents prefer it over drug therapy. It may also be used in conjunction with drug therapy if a partial response is obtained to the drugs approved by the Food and Drug Administration (FDA) or when comorbid disorders are present concomitantly. Despite all of the different behavioural therapies and non-stimulant treatments, the treatment of ADHD with psychostimulants is still the treatment of choice. However, despite their recognised efficacy and safety, their chronic use during adolescence has been criticised. In addition, 10-30% of individuals with ADHD discontinue psychostimulant treatment (Barkley 1977; Elia 1991; Wilens, 2006). For these reasons, there is no real gold standard of treatment for ADHD and therefore research into the neurobiological causes of ADHD may lead to the development of more specific and efficient treatments (drug or behavioural).

1.1.3. GENETICS AND ENVIRNOMENTAL RISK FACTORS OF ADHD

Despite diagnostic criteria and efficacious treatment for ADHD, the underlying causes of the disorder are far from clear. There is evidence to suggest a variety of risk factors, both genetic and environmental.

Human genetic studies have found ADHD to have a strong genetic component because family studies have consistently indicated a strong familial genetic contribution (Biederman et al., 1990; Tannock, 1998). The concordance rate of ADHD is 81% in monozygotic twins,

in comparison to 29% in dizygotic twins (Gilger et al., 1992). However, genetic factors in ADHD probably involve multiple genes of moderate effect. To date, no single gene has been discovered to play a major role, though several gene associations have been found. Monoamine oxidase A (MAOA) is crucial for the metabolism of monoamine neurotransmitters such as serotonin, noradrenalin, and dopamine. Genetic studies have suggested MAOA to be strongly associated with ADHD, especially the impulsivity seen in ADHD Hyperactive/Impulsive presentation, (Liu et al., 2011). Meta-analyses and pooled data analyses have proposed ADHD to be associated with polymorphisms in the dopamine receptor (DRD4 and DRD5), dopamine transporter, and serotonin transporter (Thapar et al., 2005).

Genes encoding the noradrenalin transporter (NET), and a2-adrenoceptor (Park et al., 2005; Brookes et al., 2006; Kim et al., 2006) have been found to be associated with ADHD. Furthermore, Sonuga-Barke et al. (2011) found a specific allele of SERT (SLC6A4) promoter linked to delay aversion in individuals with ADHD. In line with this, individuals with ADHD have been shown to have alterations in serotonin metabolism (Hoshino et al., 1985). Yet, due to the complexity and heterogeneity of ADHD, the results from the genome-wide scans and candidate gene experiments studied have been inconclusive (Faraone et al., 2005).

Environmental influences have been suggested to play a part in the etiology of ADHD, for example, prenatal exposure to nicotine from smoking in utero has been associated with ADHD, as well as mercury and alcohol exposure (Knopik et al., 2005; Anderson et al., 1981; Linnet et al., 2003; Thapar et al., 2003; Neuman et al., 2007; see Figure 1.1). Specific complications associated with ADHD include toxaemia or eclampsia, poor maternal health, foetal distress and low birth weight. The basal ganglia, which are commonly implicated in ADHD (see Section 1.1.4), are markedly susceptible to hypoxic insults, as they are one of the

most metabolically active areas in the brain. Small prenatal exposures of mercury, due to contamination in the maternal diet, produced negative consequences on the IQ, language development, visual-spatial skills, gross motor skills, and memory and attention in offspring, linking it to ADHD (Anderson et al., 1981). Tobacco smoke contains monoamine oxidase inhibitors. These compounds significantly decrease MAO activity in smokers (Herraiz and Chaparro, 2005), thus there is a decreased break down of monoaminergic neurotransmitters in the prenatal brain during development. Similarly, parental alcoholism has been found to increase the risk of ADHD in offspring (Knopik et al., 2005). It is clear that ADHD etiology is the complex relationship of numerous neuro-biological factors including genetic abnormalities and environmental factors. Importantly, it is the effect of these factors on key neurological systems, and most importantly their impact on the monoamine systems that seems to be critical in ADHD.

1.1.4. ALTERATIONS TO NEURAL CIRCUITRY IN ADHD

In addition to the genetic and environmental risk factors identified for ADHD, there are a number of structural abnormalities found in the brains of individuals with ADHD. This includes a reduction in total brain size that persists into adolescence (Castellanos et al., 2002) but also reduced dimensions of several brain regions (Hynd et al., 1990; 1991; 1993), which are implicated in the neural circuitry of attention. Studies in humans have supported the division of attention in to a posterior and anterior system (see Figure 1.1, Posner and Petersen, 1990). The posterior system includes the superior parietal cortex, the superior colliculus and the pulvinar nucleus. This system receives a dense innervation from the locus coeruleus (LC; Holets, 1990). Noradrenalin from the LC enhances the signal-to-noise ratio and primes, according to Pliszka et al. (1996), the posterior system to orientate to novel stimuli. Attention then shifts to the anterior system, which is known to control executive functions. It consists of the PFC and the anterior cingulate gyrus. The sensitivity of this

system is modulated by dopamine from the ventral tegmental area (VTA). According to Pliszka et al. (1996), a noradrenergic dysfunction could inhibit the priming of the posterior system and lead to attention deficits. A loss of dopamine could induce deficits in the anterior system and impair executive functions.

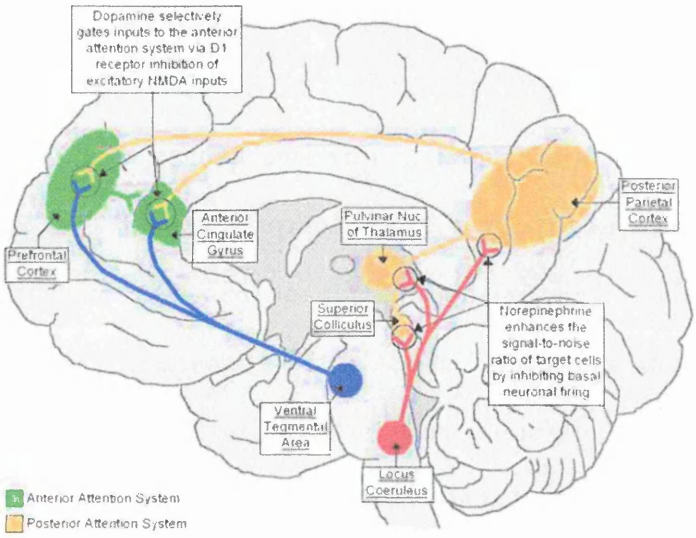

Figure 1.1: Midsagittal view of the brain illustrating Plitzska et al. (1996) multi-stage model attention. Red lines indicate noradrenergic pathways and blue lines represent dopaminergic pathways. Adapted from Himelstein et al. (2000), noreprephrine is in reference to noradrenalin, NMDA is in reference to glutamate input.

Evidence of disruption to these attentional systems in ADHD includes a reduction in the right PFC volume in children with ADHD (Castellanos et al., 2002; Filipek et al., 1997) and the fact that individuals with prefrontal lesions show behavioural characteristics similar to ADHD (Benton, 1991; Heilman et al., 1991). Basal ganglia nuclei were also found to have a reduced volume (Castellanos et al., 2002; Filipek et al., 1997) and both the basal ganglia and frontal lobe volumes correlate with the degree of impairment in attention and inhibition in children with ADHD (Casey et al., 1997; Semrud- Clikeman et al., 2000).

Several single photon emission computed tomography (SPECT) studies have shown a reduced blood flow in prefrontal regions and the connecting pathways to the limbic system and cerebellum in individuals with ADHD (Lou et al., 1984; 1989; 1990; Sieg et al., 1995).

Similarly, children with ADHD show different activation patterns during attention and inhibition tasks within prefrontal regions, basal ganglia and cerebellum (Rubia et al., 1999; Teicher et al., 2000; Vaidya et al., 1998; Yeo et al., 2003). Therefore, in ADHD, there appears to be a functional disturbance within the frontostriatocerebellar system affecting the neurotransmitters dopamine and noradrenalin. These disturbances may be associated with genes regulating dopaminergic, noradrenergic and probably serotonergic functions (Castellanos and Tannock, 2002). The exact nature of the neurotransmitter dysfunctions is not clear but a number of studies have investigated these, some of which are described next.

1.1.5. NEUROCHEMISTRY OF ADHD

Dopamine

As described above, dopamine is one of the key neurotransmitters in the anterior attention system and is strongly implicated in ADHD. Alterations have been found in both pre and post synaptic dopamine function in ADHD and this has led to the development of the 'Dopamine Hypothesis of ADHD'. This hypothesis states that ADHD symptoms are associated with both hyper- and hypo-function of dopamine. The central nigrostriatal, mesolimbic and mesocortical dopamine systems (Dahlstrom and Fuxe, 1964, see Figure 1.2) are thought to be implicated in the expression of ADHD, producing altered reinforcement and extinction processes, as well as 'extrapyramidal' symptoms, such as difficulty controlling motor activity (Volkow et al., 2001). Castellanos (1997) suggested that in dopamine presynaptic receptor rich areas, such as in subcortical regions, presynaptic effects predominate, and thus, cause a decrease in synaptic dopamine. While in cortical areas with sparse presynaptic receptors, the postsynaptic effects prevail, producing increased synaptic dopamine. An example of this is the association of ADHD with relative over-activity of the nigrostriatal pathway. The dopaminergic neurons of the substantia

nigra and the adjacent VTA project to the striatum and to regions in the neocortex (see Figure 1.2). They are important in the initiation of movements and for emotional processes and are tightly regulated by inhibitory autoreceptors, as well as by long-distance feedback from the cortex.

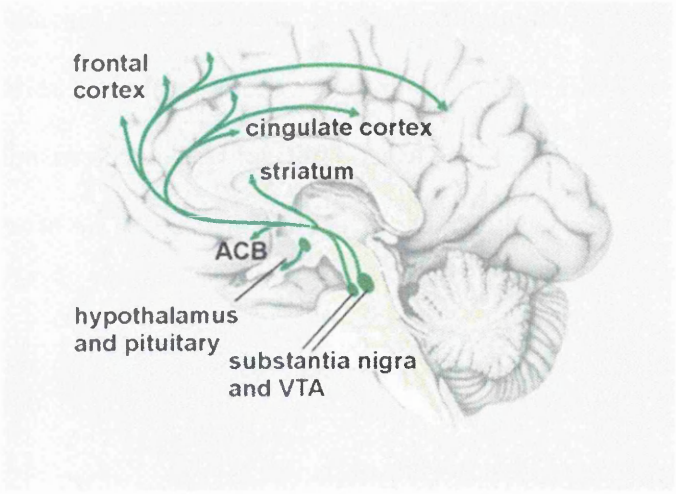

Figure 1.2: The key neuron innervation of dopamine. ACB: nucleus accumbens, VTA: ventral tegmental area. Adapted from Fuchs and Flügge (2004).

One of the key factors that support a role for dopamine in ADHD neurobiology is the effectiveness of psychostimulant drugs which act on the dopamine system. Levy (1991) proposed that psychostimulant treatment corrects an underlying dopamine deficiency, increasing the effect of impulse-associated release of dopamine (Suaud-Chagny et al., 1989). Others proposed that psychostimulants function as antagonists (Solanto, 2002) by raising background levels of dopamine, which then suppresses release of dopamine by acting on autoreceptors (Seeman and Madras, 1998). In support of the role of dopamine in the therapeutic effects of psychostimulants, Volkow et al. (1998) showed that a standard clinical dose methylphenidate would block about 60% or more of dopamine transporter (DAT) and clinically relevant doses of methylphenidate produce their therapeutic effects by increasing extracellular dopamine (Volkow et al., 1999; 2002a; Rosa Neto et al., 2002). Consistent with this, a significant association was found between extracellular brain dopamine levels and the motivation to undertake a mathematical task (Volkow et al., 2004),

leading the authors to postulate that methylphenidate's therapeutic effects may make stimuli more motivationally salient and therefore improve performance, by its ability to enhance stimuli-induced dopamine increases.

Noradrenalin and serotonin

Noradrenalin is one of the key neurotransmitters in the posterior attention system and is strongly implicated in ADHD. Similarly, psychostimulants used for the treatment of ADHD have been found to work on dopamine and noradrenalin, as well as serotonin at higher doses (see Section 1.1.2). Growing evidence in recent years shows strong evidence that disturbances within the fronto-striatal system and altered levels of all monoamines, such as noradrenalin and serotonin (therefore not only dopamine), are involved in the pathophysiology of ADHD (see Figure 1.2 and 1.3 for the innervation patterns of these monoamines). The noradrenergic neurons of the locus ceruleus project to the limbic and cortical regions, and to the thalamus, cerebellum, and spinal cord. They play an important role in the regulation of mood and attention (see Section 1.1.4). The serotonergic neurons located in the raphe nuclei project to almost all parts of the brain and are involved in many functions including the regulation of emotional processes. Similarly preferential activation of PFC catecholaminergic neurotransmission has been shown to play a pivotal role in the therapeutic actions of low-dose psychostimulants in the treatment of ADHD (Berridge et al., 2006).

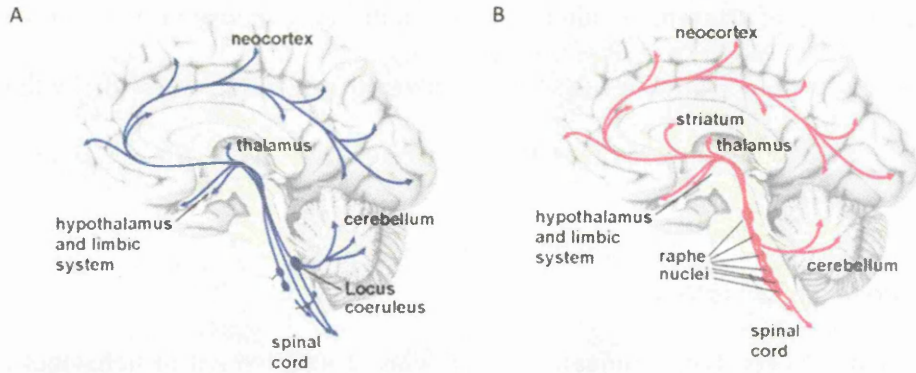

Figure 1.3: The noradrenalin and serotonin key neuron innervation, A: Noradrenalin; B: Serotonin. Adapted from Fuchs and Flügge (2004).

An alternative theory to dopamine mediating the therapeutic effects of psychostimulants on ADHD is that the therapeutic effects are mediated by noradrenalin (Pliszka et al., 1996; Arnsten, 2006). Atomoxetine does increase dopamine and noradrenalin in prefrontal cortex regions because the noradrenalin transporter plays an important role in clearance of dopamine in this region (Bymaster et al., 2002; Swanson et al., 2006), which has been shown to play a key role in attention and higher cognitive processes (Bymaster et al., 2002). In contrast to methylphenidate, atomoxetine does not increase dopamine in striatum or limbic areas (Bymaster et al., 2002), although evidence from animal studies suggests an increase in prefrontal dopamine (rat: Swanson et al., 2006; mouse: Kodo et al., 2010), which may indirectly alter subcortical dopamine activity (Deutsh, 1992). Therefore with both atomoxetine and fluoxetine having some efficacy (Barrickman et al., 1991; Gibson et al., 2006), and being largely devoid of activity of the mesolimbic dopamine system linked to substance abuse (Pettersson et al., 2011), it seems likely that such activity is not essential.

In summary, even though the neurobiology of ADHD is not fully understood, dopamine, noradrenalin and serotonin transmission are likely to be affected and this will have significant consequences in the attentional circuitry of the brain as well as other regions implicated in a variety of functions.

1.1.6. UNIFYING THEORIES OF ADHD

There have been a number of attempts made to develop a unifying theory of ADHD based on the symptoms and known neurobiology described above. However, it is noteworthy that despite the accepted role of other monoamines, focus still remains primarily on dopamine.

Impairment in behavioural inhibition

The first unifying theory to receive significant support placed impairment of behavioural inhibition at the centre of the disorder (e.g., Barkley, 1997; Nigg, 2001; Sergeant et al.,

2002). It is speculated that individuals with ADHD have a reduced ability to inhibit unnecessary or inappropriate behavioural responses and that this may result in a number of secondary effects. Barkley (1997) outlines how behavioural inhibition can relate to four distinct executive functions as shown in Figure 1.4, which he suggests are affected in ADHD.

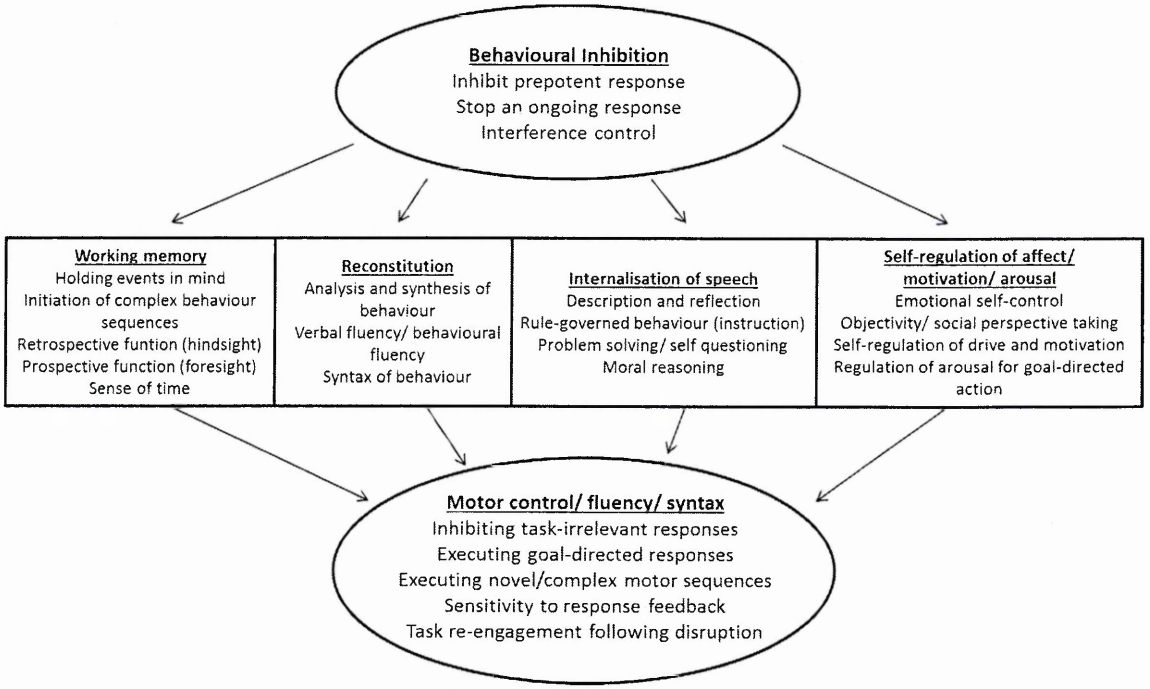

Figure 1.4: A schematic configuration of a conceptual model that links behavioural inhibition with the performance of the four executive functions that bring motor control, fluency, and syntax under the control of internally represented information. Adapted from Barkley (1997).

There are a number of direct measures of behavioural inhibition, for example, children with ADHD talk more than other children, whether to others (Cunningham and Siegel, 1987) or out loud to themselves (Berk and Potts, 1991), which could suggest poor behavioural inhibition. Further evidence of poor inhibition in ADHD comes from studies that use motor inhibition tasks, such as Go-No-Go paradigms (Iaboni et al., 1995; Milich et al., 1994). One of the most commonly used methods to investigate behavioural inhibition is occulomotor paradigms which have been used to assess and localise dysfunction of cognitive control in individuals with ADHD. These paradigms examine functions necessary for the planning and the execution of saccades, such as motor response preparation, response inhibition, and working memory. Interestingly, such paradigms are dependent on the superior colliculus, an area of the midbrain critical for saccade generation and yet, until recently, overlooked in

ADHD research (Overton, 2008). Research using such tasks has shown that, compared to age matched controls, children with ADHD have significantly longer saccade latencies and duration for:

- Visually guided saccades (VGS), an assessment of sensorimotor function such as orientation of attention and oculomotor response preparation (Mahone et al., 2009; Goto et al., 2010),
- Memory guided saccades (MGS), an assessment of working memory function (Goto et al., 2010).
- Prosaccades, an assessment of stimulus initiated reflexive responses (Klein et al., 2003; Munoz et al., 2003).
- Antisaccades, an assessment of inhibitory function of stimulus initiated reflexive responses. (Munoz et al., 2003; Feifel et al., 2004; Karatekin, 2006; Karatekin et al., 2010).

Additionally, children with ADHD also show significantly more anticipatory errors (Ross et al., 1994; Mostofsky et al., 2001; Goto et al., 2010) and deficits in accuracy (Ross et al., 1994; Mostofsky et al., 2001; Goto et al., 2010). A significantly higher percentage of direction error rates are also found in individuals with ADHD, who have greater difficulty in suppressing unnecessary reflexive saccades (Munoz et al., 1999; Ross et al., 1994; Mostofsky et al., 2001; Klein et al., 2003; Goto et al., 2010), as well as a greater intra-subject variance (Mostofsky et al., 2001; Munoz et al., 2003) in these tasks compared to controls. Interestingly, other researchers using similar oculomotor paradigms found no differences between children with ADHD and their age matched controls in the VGS task (Loe et al., 2009), and in the MGS task (Loe et al., 2009; Mahone et al., 2009).

There are a variety of possibilities for the inconsistences in results, for example the inter-individual variability of the disorder between individuals and especially between different presentations or subtypes (Sagvolden et al., 2005). One possibility may be due to

differences in the levels of arousal in the individuals examined. In support of this, Karatekin (2006) found differences between the ADHD group and the control group when the antisaccade task was novel. The individuals with ADHD were found to be comparable to individuals without ADHD on the second administration of the same task. This suggests that increased antisaccade response times in ADHD may be associated with attentional deficits and amending arousal levels on a novel task, rather than individuals with ADHD having an ongoing reduced ability to inhibit unnecessary or inappropriate behavioural responses.

According to Barkley (1997), poor behavioural inhibition will lead to secondary deficiencies in four key areas; working memory and its subfunctions (as found by Zentall and Smith, 1993), self-regulation of affect/motivation/arousal, internalisation of speech, and reconstitution. Researchers have frequently commented on ADHD being associated with less drive, motivation, or effort in the performance of goal-directed behaviours when performing repetitive tasks that involve little or no reinforcement (Barkley, 1990; Antrop et al., 2006; Sonuga-Barke et al., 1992; Marco et al., 2009). Children with ADHD have impaired reconstitution abilities such as language fluency, compared with those without ADHD, appear to produce less speech in response to confrontational questioning (Tannock, 1996), and are less competent in verbal problem-solving tasks (Douglas and Parry, 1983). Studies have also found immaturity in self-directed speech, and moral reasoning in children with ADHD (Berk and Potts, 1991; Rosenbaum and Baker, 1984). Thus, all four distinct executive functions outlined by Barkley (1997) are present in children with ADHD and conform to poor behavioural inhibition in this disorder. Research indicates that the ability to interrupt an about-to-be-executed response requires activation of the right inferior frontal cortex (Aron et al., 2003), as well as regions in the basal ganglia, including the caudate (Casey et al., 2002). Interestingly, both basal ganglia and frontal lobe volumes correlate with the degree of impairment in attention and inhibition in children with ADHD (Casey et al., 1997; Semrud- Clikeman et al., 2000).

Enhancing signal/noise ratio in attention involves the noradrenergic ascending pathway from the locus coeruleus to the cortex (Pribram and McGuinness, 1975; Tucker and Williamson, 1984; again see Strelau, 1994), and is key to the preliminary stages of processing information (Sergeant et al., 1999; Tucker and Williamson, 1984). Phasic pupillary dilations are relatively direct measures of recruitment of cognitive resources in accordance with task demands (Karatekin, 2007). In an antisaccade task, analysis of pupillary dilation data found no differences between groups, suggesting that allocation of effort was comparable in children with ADHD and healthy controls, and the ADHD group had the same level of arousal during the task than the control group. The ADHD group and controls also showed differences in the parameters of erroneous antisaccades in comparison to regular prosaccades, thus the children with ADHD were attentive to the instruction to make antisaccades, and were not simply making prosaccades. These comparisons suggest that the results were not due to difficulties with goal neglect in children with ADHD (Karatekin, 2007), and in adults (Carr et al., 2006; Feifel et al., 2004; Nigg et al., 2002), thus, the study questions the hypothesis that inhibition is a core cognitive impairment of ADHD (Fischer et al., 2005). In summary, oculomotor paradigms have contributed to the understanding of the pathophysiological basis of ADHD, yet have yielded inconsistent results.

Neural systems including the basal ganglia, the limbic system, the thalamus, and the prefrontal cortex underlie executive control and arousal (Barkley., 1997; Gray and McNaughton., 1996). Attention and impulsive control continue to develop throughout childhood, seemingly due to ongoing myelination and increased development of frontal cortical neural networks (Benes, 2001). Children aged 8-11 years old show an increased ability to ignore competing stimulus-driven responses (Huang-Pollock et al., 2002), with the faculties to suppress pre-potent responses continuing to develop during adolescence

(Bedard et al., 2002). Therefore, the combination of dysfunction, and slower development of "top-down" executive control processing (e.g., suppressing competing responses) and "bottom- up" motivation or regulation processing (e.g., arousal, activation, or delay–reward gradient) is linked to the etiology of ADHD.

The dynamic developmental theory

A second unifying theory that has received widespread support is the dynamic developmental theory of ADHD devised by Sagvolden et al. (2005). This theory indicates that there may be two core behavioural processes causing the overall symptoms of ADHD: altered reinforcement of novel behaviour, as measured by a delay-of-reinforcement gradient, and defective extinction of formerly reinforced behaviour, which could cause the delay aversion, development of hyperactivity, impulsiveness, deficient sustained attention, increased behavioural variability, and disinhibition seen in ADHD.

Delay-of-reinforcement gradient is the relationship between the effect of the reinforcer and the intermission separating the response and reinforcer (Johansen et al., 2002). It has been proposed that children with ADHD have an altered reinforcement pattern (Sagvolden et al., 1998). Altered reinforcement processes in ADHD can be depicted as a narrower time window than normal for associating behaviour with its consequences, or theoretically by a shorter and steeper delay-of-reinforcement gradient (Figure 1.5). It has been suggested that this altered reinforcement process may define an ADHD endophenotype, a hereditary characteristic that is normally associated with a condition but is not a direct symptom of that condition. It has been argued that the key features of ADHD: deficient sustained attention, hyperactivity, and impulsiveness, may all be due to altered reinforcement mechanisms and a shortened delay-of-reinforcement gradient (Sagvolden and Sergeant, 1998).

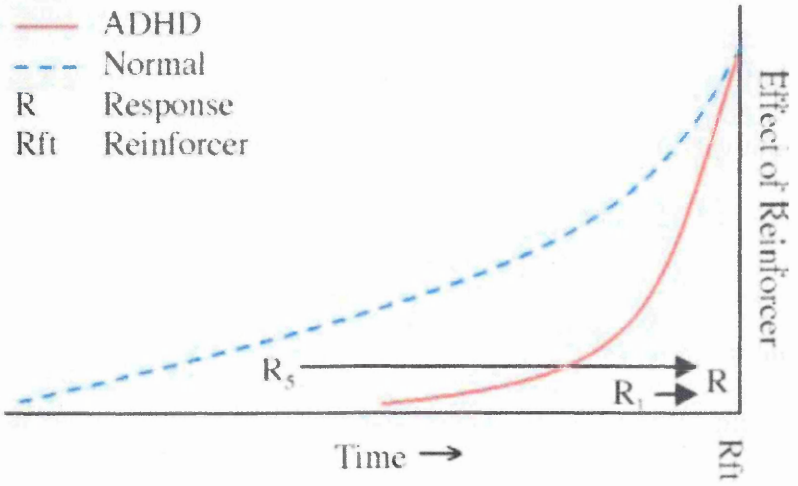

Figure 1.5: Theoretical delay-of-reinforcement gradients. The shorter and steeper delay gradient for ADHD (solid red line) means that the association between the response R5 and R will not be reinforced, unlike with a normal delay gradient (broken blue line). The relationship between R1 and R is near enough to be reinforced. Adapted from Sagvolden et al. (2005).

A short and steep delay-of-reinforcement gradient implies that reinforcement should be immediate to be effective, yet this effect becomes less efficient as the delivery of the reinforcer is delayed (Sagvolden et al., 2005). Therefore, if children with ADHD have an altered reinforcement mechanism, as is demonstrated by their steeper delay-of-reinforcement gradient, reinforcers presented immediately will be more effective (Sagvolden et al., 1998). Most behavioural treatment programs which have been found to be effective for children with ADHD have included increased frequency of reinforcement and improvement of self-control (see Section 1.1.2, e.g., Barkley, 1998).

In addition to this evidence for an altered delay-of-reinforcement gradient, there is also significant data supporting altered extinction behaviour, already discussed in Section 1.1.1. These two core behavioural processes are thought to be primarily linked with hypofunctioning of the mesolimbic (limbic loop) dopamine system (Johansen et al., 2002, see Figure 1.7). However, Sagvolden et al. (2005) suggest this hypofunction affects three distinct loops, shown in Figure 1.6. A hypofunctioning mesocortical (prefrontal loop) dopamine system is associated with attention response deficiencies (deficient orienting

responses, impaired saccadic eye movements, and poorer attention responses toward a target) and poor behavioural organisation. It is thought that a hypofunctioning nigrostriatal (motor loop) dopaminergic system impairs the modulation of motor functions and causes deficiencies in non-declarative habit learning and memory. Problems such as these will cause deficiencies, such as disinhibition of responses when quick reactions are needed.

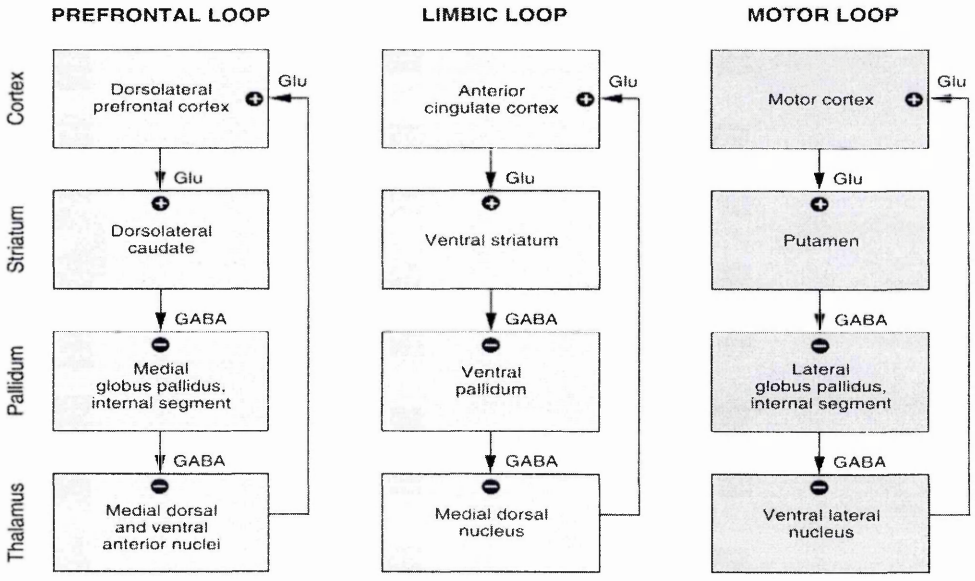

Figure 1.6: Dopamine hypofunction affecting three distinct loops. The figure illustrates that there are several circuits affected probably with distinct functions. Adapted from Sagvolden et al. (2005).

Dopamine can be considered as operating in two distinct components - the phasic and tonic (Grace, 2001). It has been hypothesised that the phasic component represents a reinforcer prediction error signal (Schultz, 2002), although there is also a suggestion that it more broadly represents salient stimuli (Redgrave et al., 2008). This phasic dopamine response has been shown to be modulated by sensory information transmitted to the dopamine neurons via the superior colliculus (Dommett et al., 2005; Coizet et al., 2006). This dopamine response effectively allows only the most powerful signals to be transmitted through the striatum to the pallidum (Schultz, 2002), and thus, it has potent regulatory control (Grace, 2002). The tonic dopamine is known to control the phasic dopamine release via synthesis- and release-modulating autoreceptors on the dopamine terminals. Normally, the tonic dopamine level is too low to stimulate postsynaptic dopamine receptors but

rather acts to down-modulate action potential induced phasic dopamine release (Grace, 2001; 2002). Sagvolden et al. (2005) suggest that ADHD is associated with a dysregulation of tonic/phasic dopamine control, giving rise to stunted phasic dopamine responses (Russell et al., 1995) despite low tonic dopamine levels.

In healthy individuals, research has shown that immediately subsequent to a reinforcer there is a phasic augmentation of dopamine activity (Schultz, 2002; Waelti et al., 2001), and therefore release of dopamine in association with reinforcement. From the midbrain to the striatum and the frontal cortex a transient peak of extracellular dopamine concentrations occurs as dopamine is released through a burst of phasic dopamine neuron activity subsequent to a reinforcer (Schultz, 2002). Therefore this peak appears to be associated with stimuli that function behaviourally as reinforcers, and thus, are salient stimuli. The phasic burst activity ensues after a reinforcer occurs if the reinforcer delivery differs from the animal's previous behavioural relationship, such as when the reinforcer is delivered during novel behaviour, or delivered at an unusual time, or when a higher-than-usual reinforcing value is placed on the reinforcer (Schultz, 2002; Waelti et al., 2001). Additionally, a short-lasting phasic depression of tonic dopamine neuronal activity occurs when formerly recognised stimulus–response–reinforcer relations are terminated (Schultz, 2002; Waelti et al., 2001) i.e. the extinction process. This depression also occurs with reinforcers that have a decreased reinforcer value compared to that previously experienced.

Sagvolden et al. (2005) propose that if children with ADHD have an overall reduced tonic dopamine level in comparison to healthy children, for normal reinforcement to occur, an increased phasic release of dopamine is needed to generate the postsynaptic modulations required. Likewise, a less effective reinforcement in ADHD will occur, if the child has a normal tonic dopamine level, but reduced phasic dopamine release related to a reinforcer.

An elevated reinforcer value is necessary to normalise the reinforcement process in both circumstances, and thus, strengthened, more salient reinforcers are essential to normalise the behaviour seen in children with ADHD (Sagvolden et al., 2005). In support of the dynamic developmental theory, psychostimulant treatments have been shown to extend the length of the delay-of-reinforcement gradient (Sagvolden et al., 1988), and generate an amplified reinforcement prediction error signal. Furthermore, Schultz (2002) suggests that pyschostimulant application causes substantial behavioural changes because they produce a powerful focusing signal generating modifications in synaptic transmission.

In addition to the theory providing an account of behavioural symptoms and possible therapeutic mechanisms, it also allows for the gene-environment interaction thought to be important in the etiology of ADHD (see Figure 1.7). Sagvolden et al. (2005) posit that during the early stages of ADHD development various toxins and genetic influences could cause unsuitable overactivity of mesolimbic ventral tegmental dopamine neurons, thus augmenting excitatory synaptic transmission, causing an increased dopamine release. This could result in depolarisation block of ventral tegmental dopamine neurons and hypoactivity of the mesolimbic dopaminergic system, due to a decrease in synaptic strength, and thus a long-term depression in this area. Insufficient glutamate input from the prefrontal cortex to dopamine neurons, as well as an imbalance in noradrenalin and serotonin systems have also been suggested to play a role in the dopaminergic imbalance (Sagvolden et al., 2005).

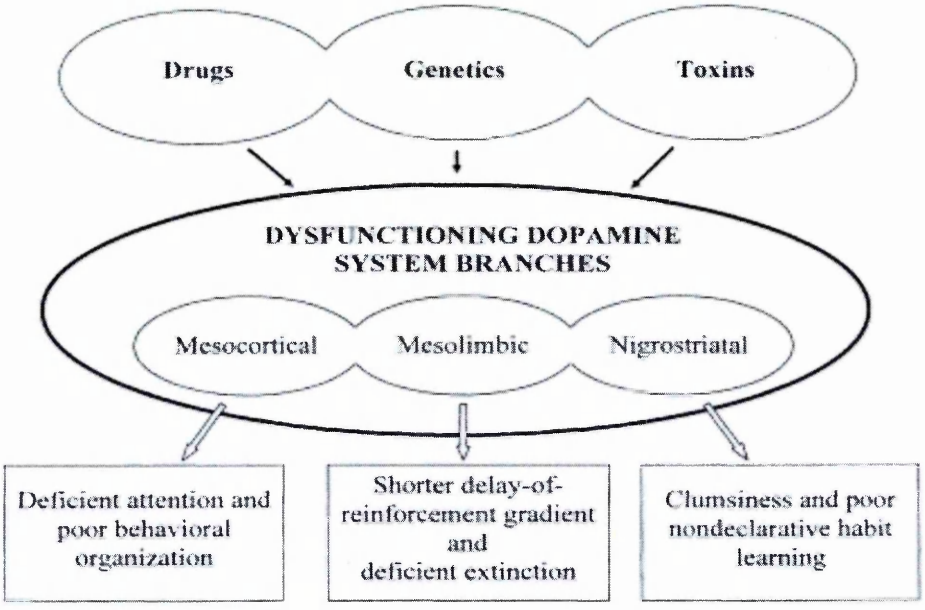

Figure 1.7: Dysfunction of dopaminergic systems resulting from drug abuse, genetic transmission, or environmental pollutants may cause ADHD symptoms by interacting with frontostriatal circuits (not shown). Adapted from Sagvolden (2005).

Irrespective of which unifying theory is accepted, a role for dopamine is crucial. However, it could be that the dysregulation of dopamine is a secondary effect of a dysfunction in the initial processing of salient reinforcers, where the saliency of stimuli has been dampened. If this were the case, changes upstream of the dopamine neurons should be found that would be capable of influencing their activity. A candidate structure for this is the superior colliculus, which has direct connections to the dopamine neurons mentioned (Comoli et al., 2003; McHaffie et al., 2006) and has been shown to be capable of activating and modulating midbrain dopamine neuron phasic activity (Dommett et al., 2005; Coizet et al., 2006), which could lead to the secondary dysregulation of dopamine suggested in both theories.

1.2.THE SUPERIOUR COLLICULUS

The superior colliculus (SC) is one of the most ancient regions of the vertebrate central nervous system. In this area, broad neural circuits, including afferents from numerous sensory pathways converge, enabling the SC to be a key area for primary sensory

processing, multi-sensory integration and the generation of motor commands for orientation behaviours. It is highly conserved in vertebrates, referred to as the SC in mammals (see Figure 1.8), and closely resembles its homologue the optic tectum in birds, fish and amphibians (Gaither and Stein, 1979).

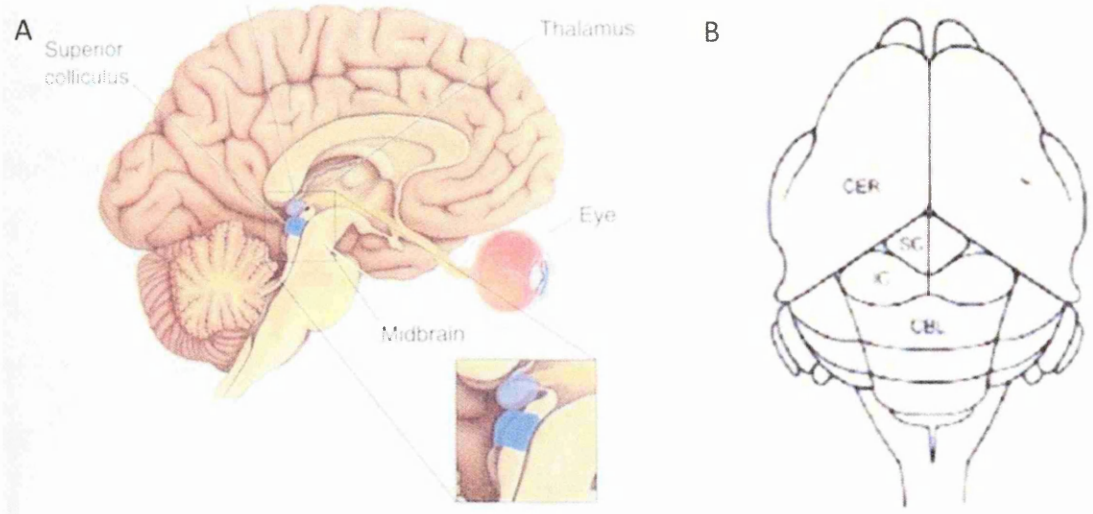

Figure 1.8: A diagram of the location of the Superior colliculus in A: the human brain (Adapted from Morén et al., 2013); and B: the rat brain, CER: cerebral cortex; SC: superior colliculus; IC: inferior colliculus; CBL: cerebellum (Adapted from Kiernan, 2008).

1.2.1. THE ANATOMY OF THE SUPERIOUR COLLICULUS

The SC is a laminar structure located on the dorsal surface of the midbrain (Huerta and Harting, 1984), ventral to the cortex and surrounding the dorsal aspect of the periaqueductal grey (PAG). It is organised into seven alternating cellular and fibrous dorsoventral layers (Kanaseki and Sprague, 1974), but the structure is commonly divided operationally into two parts: the superficial layers (layers I-III), concerned with visual processing, and the deeper layers (layers IV-VII), concerned with multimodal processing and motor activity. The superficial layers run from the Zonal Layer (Zo), through the Superficial Grey (SuG), to the Opticum (Op), while the deep layers from dorsal to ventral are

the Intermediate Grey (InG), Intermediate White (InWh), Deep Grey (DpG) and Deep White (DpWh; see Figure 1.9).

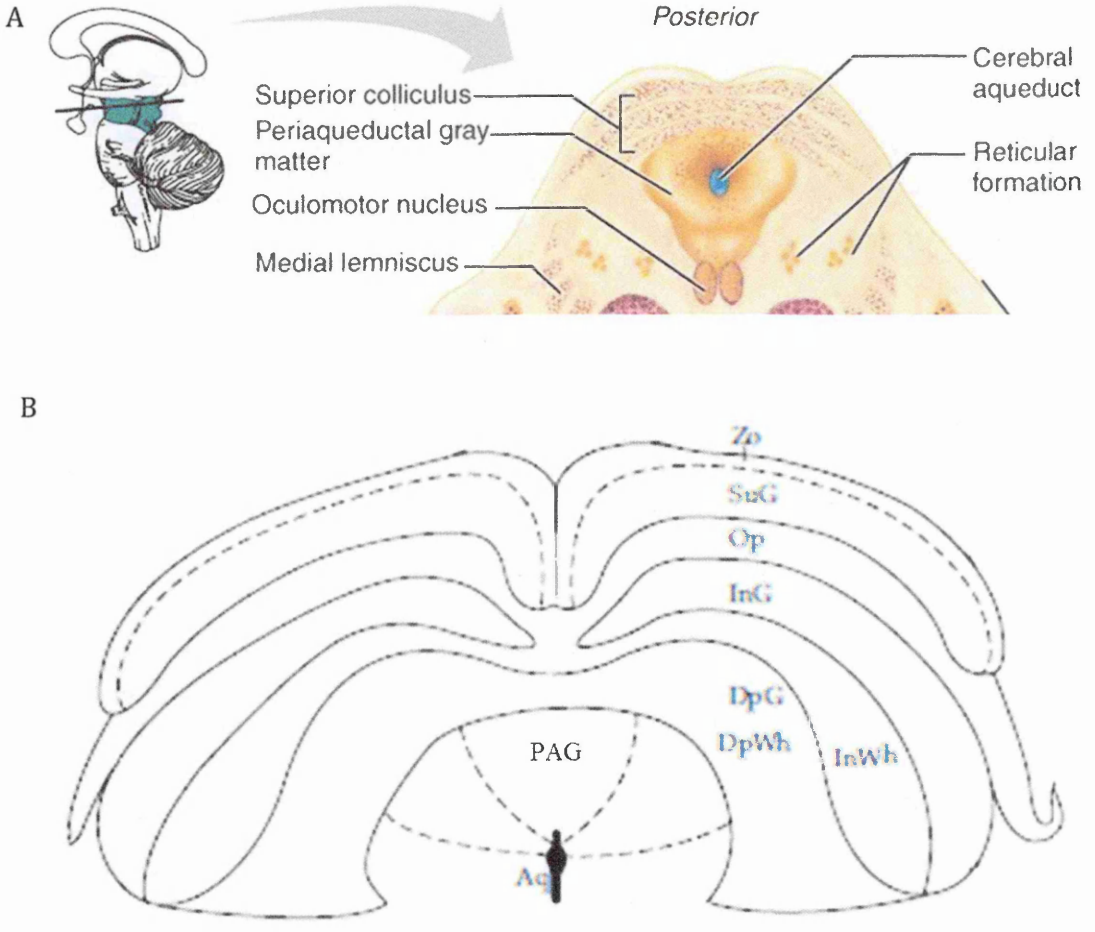

Figure 1.9: A: A transverse section through the human brain of the superior colliculus (Adapted from Cummings, 2001); B: A coronal section through the rat brain of the superior colliculus at -6.3 mm from Bregma . The three superficial layers; and the four deeper layers; Zo: zonal layer; SuG: Superficial grey; Op: Opticum; InG: Intermediate grey; InWh: Intermediate white; DpG: Deep grey; DpWh: Deep white; PAG: Periaqueductal grey; Aq: cerebral aqueduct (Adapted from Paxinos and Watson, 1998).

Historically the superficial and deep layers of the SC have been considered as separate structures with the basis of the divide being the differences in neuronal morphology, afferent-efferent projections, physiological properties, and behavioural correlates (Edwards, 1980). However, it is clear that there is an interaction between them. Anatomical

connections between SuG and InG have been demonstrated in hamsters (Mooney et al., 1984; Mooney et al., 1988) and physiological studies (Mooney et al., 1992) indicate that these connections carry visual information to the deep SC. Following anatomical reconstructions of SC neurons in the platyrrhine (old world monkey) species, axons of superficial layer neurons were shown to project to the deeper layers of the SC, and dendrites of motor neurons within the deeper layer connect with the superficial layers (Moschovakis et al., 1988). Helms et al. (2004) verified that stimulation of the superficial layers evoke excitatory postsynaptic currents in intermediate layer premotor cells, presenting compelling evidence for an influential, monosynaptic, excitatory pathway connecting these layers. Isa et al. (1998) also showed that these responses can be blocked by bath application of AP5 (NMDA receptors) or CNQX (AMPA/kainite receptors) indicating their glutamatergic nature and their mediation by both NMDA and non-NMDA receptors. Therefore, these findings show that regarding these regions as separate structures is no longer functionally and physiologically sufficient.

1.2.2. THE CONNECTIONS OF THE SUPERIOR COLLICULUS AND THEIR PROPERTIES

Neurons in the superficial layers integrate visual information from the retina, cortex and other sources, while the deep layers incorporate information from a diverse array of cortical and sub-cortical sensory areas, including the superficial layers, to generate motor commands.

Visual/ superficial layer afferents

The superficial layers of the SC are exclusively concerned with the processing of visual information, having major connections with structures that analyse visual information. The receptive fields of superficial layer neurons form a retinotopic map of the contra-lateral visual space across the dorsal surface; receiving major afferents from the contralateral retina terminating in the SuG (Hendrick et al., 1970; Tigges and Tigges, 1970; Hubel et al.,

1975). In mammals, direct inputs from visual forebrain areas connect to the superficial layers of the SC, approximately a third of its cortical afferents arise from Layer V of the visual cortex (Collins et al., 2005) which enter from the rostro-medial pole and travel along the Op to terminate in the SuG (Lund et al., 1975). In addition to receiving inputs originating in the extrastriate visual cortical areas (Wurtz and Albano, 1980; Boehnke and Munoz, 2008), the superficial layers of the SC also receive direct inputs from cortical eye fields, including the frontal eye field (FEF; Bruce and Goldberg, 1985). In many cases afferents from the retina and visual cortex synapse on the same neurons allowing almost raw visual information from the retina to converge with processed information from the lateral geniculate nucleus (LGN) and visual cortex.

Multimodal/ deep layers afferents

The number of structures projecting to the deep layers is substantially greater than found for the superficial layers. Edwards et al. (1979) demonstrated more than 40 sources of subcortical afferents to the intermediate and deep layers of the cat. Large sensory neurons in InG receive subcortical projections from almost all areas of the brainstem as well as key structures in the auditory, somatosensory and nociceptive pathways (Karten et al., 1973; Wurtz and Albano, 1980; Knudsen and Knudsen, 1983; Stein and Meredith, 1993; King et al., 1998). Also terminating in the deep SC are sensory projections from many cortical areas including a major projection from the anterior ectosylvian sulcus which carries multi-modal information. Sensory neurons in the deep SC often respond to sensory stimuli of several modalities and the presentation of multi-modal stimuli yields responses which can be either facilitatory or inhibitory depending on the spatial and temporal correspondence properties of their individual modality components. Furthermore, spatial representations of the visual, auditory and somatosensory worlds are aligned in the deep SC and maintained in register with the retinotopic organisation of visual receptive fields in

the superficial layers (Chalupa and Rhoades, 1977; Drager and Hubel, 1976; Stein et al., 1976).

Unlike the superficial layers of the SC, relatively little direct input to the deeper layers arise from the retina (Berson and McIwain, 1982; Mize, 1983a; 1983b), yet many of their sensory neurons respond to vision. The majority of the visual input to the deeper layers arises from extrastriate visual regions. Other structures transmit relatively limited visual inputs to the deeper layers, including the ventral geniculate nucleus (Edwards et al., 1979; Huerta and Harting, 1984). Therefore, visual responses in the deeper SC depend profoundly on descending control from cortex. Furthermore, it is worth noting that the excitatory corticotectal inputs to superficial and deeper layer visual neurons arise from different regions of the cortex. Superficial neurons, but not deeper layer visual neurons, were depressed following deactivation of the visual cortex. By contrast, deactivation of the extrastriate visual cortex (lateral suprsylvian, LS) caused a depression on deeper layer neurons, but not superficial layer neurons (Ogasawara et al., 1984).

The deep layers of the SC receive auditory information from the auditory cortex originated from the dorsal part of the ipsilateral auditory cortical area, in layer V (Druga and Syka, 1984). However, there are a number of ascending auditory inputs (primarily contralateral), comprising of the branchium of the inferior colliculus, the external nucleus of the inferior colliculus, the nucleus sagulum, the dorsomedial periolivary nucleus, and a region medial to the trapezoid body (Edwards et al., 1979). In contrast to the visual projections, in the superficial layers, the ascending auditory inputs are much denser in the deeper layers of the SC, with the most powerful projections from the dorsal nucleus of the lateral lemniscus (Druga and Syka, 1984).

Excitatory corticotectal control is required to ensure that SC activity in both the superficial and deeper layers can be modified and modulated by experience and current needs. Removal of this corticotectal input depresses all sensory modality responses, yet there are different dependencies among sensory representations in the SC on ascending and descending inputs. Visual responses are far more depressed by cortical deactivation than are somatosensory, and somatosensory responses are far more depressed than are auditory (Stein, 1993).

The deeper layers of the SC also have several important non-sensory inputs including major inhibitory projections from the substantial nigra and zona incerta (Appell and Behan, 1990; Ficalora and Mize, 1989). The substantia nigra pars reticulata (SNr), a component of the basal ganglia, connects to the majority of contralateral SC output neurons (Moschovakis and Karabelas, 1985) through the inter-collicular commissure connecting the left and right SC (Wallace et al., 1989; 1990), and plays a crucial role in the oculomotor control, as well as other attentional and orientation behaviours (Hikosaka and Wurtz, 1983; Chevalier and Deniau, 1987).

Excitatory cortical inputs derived from the ipsilateral cortex (Ogasawara et al., 1984) are counterbalanced by contralateral inhibitory inputs (Hoffmann and Straschill, 1971; Goodale, 1973; Saraiva et al., 1978) derived from substantia nigra pars reticulata. An example of the effects of these two strong excitatory and inhibitory influences on the SC can be demonstrated in an experimental effect referred to as the Sprague effect (Sprague, 1966). A lesion to the right cortex, and thus, the removal of the strong excitatory influence on the right SC produced profound visual neglect of the left, or contralateral visual field because the right SC became dominated by inhibitory inputs from the substantia nigra pars reticulata. However, following removal of the SC on the side opposite the cortical lesion (i.e. the left SC, Sprague, 1966; Hardy and Stein., 1988) or cutting the commissure (Sherman,

1974a), eliminates these commissural-mediated inhibitory inputs. The defect was reversed by restoring a balance to the system and allowing the right SC to regain some of its function. This also highlights the important capabilities of the SC without either of these influences, because it demonstrated that the SC still functioned well enough to undergo gross attentive and orientation responses.

Excitatory deeper layer inputs also arise from the deep nuclei of the cerebellum, such as the medial and posterior interposed nuclei, and from the related perihypoglossal nucleus (Edwards et al., 1979; Kawamura et al., 1982; May et al., 1990). Convergence onto the same neurons in the deeper layers of the SC from the inhibitory input arising in the SNr (Hikosaka and Wurtz, 1989) and the excitatory input from the cerebellum (Niemi-Junkola and Westby, 2000) allows the deeper layers of the SC to have intrinsic inhibitory processes focusing the cerebellar activation on activities that are appointed for by the basal ganglia, whilst simultaneously inhibiting others. The role of this converging input is thought to help mediate the responses to novel stimuli in the environment.

In mammals, additional inputs arise from the lateral intraparietal cortex (LIP) (Paré and Wurtz, 1997; 2001; Ferraina et al., 2002) and in the dorsolateral prefrontal cortex (DLPFC) (Lynch et al., 1985), which play an important role in saccade-related activity (Johnston and Everling, 2006). Major movement-related inputs arise from gaze control areas of the forebrain. Motor afferents arising ipsilaterally directly connect the FEF with the SC in the cat and primate (Astruc, 1971; Kunzle and Akert, 1977; Kawamura and Konno, 1979; Leichnetz et al., 1981; Stanton et al., 1988). Similarly, a direct connection between motor cortices and the SC occurs in rodents (Leonard, 1969). This arrangement allows the drawing together of the many separately processed aspects of information about a single event in order to synthesise the most appropriate motor response.

Visual/ superficial layer efferent

The superficial layers have efferent connections with visual processing structures in the thalamus, and can relay the visual signals to the extrastriate visual cortex through these connections (Mohler and Wurtz, 1977; Rodman et al., 1990). In mammals, these thalamic nuclei include the lateral geniculate nucleus (LGN) and the inferior pulvinar (PULi) (Harting et al., 1973), connecting to the striate and extrastriate cortical areas (Kaas and Lyon, 2007; Berman and Wurtz, 2008; Boehnke and Munoz, 2008). For example, a pathway from the superficial layers of the SC through the PULi, predominantly within and adjacent to the medial subdivision of this structure, transmits outputs to the visual motion area of the cortex, the middle temporal area (V5/MT) (Schmahmann and Pandya, 1990; Clower et al., 2001; Berman and Wurtz, 2010; Lyon et al., 2010; Berman and Wurtz, 2010; Berman and Wurtz, 2011). This pathway is thought to play a major role in blindsight (Kato et al., 2011), and conveys suppression of saccadic activity from the superficial SC (Berman and Wurtz, 2011). The superficial layers of the SC connect to the basal ganglia via ascending projections to the thalamus and then forward to the striatum, the primary input to the basal ganglia (McHaffie et al., 2005).

Multimodal/ deep layers efferent

The projections from the deeper layers are more extensive than the efferents from the superficial layers. There are two large descending pathways, travelling to the brainstem and spinal cord, and numerous ascending projections to a variety of sensory and motor centres, including several that are involved in generating eye movements. The SC projects extensively to the forebrain network, influencing the DLPFC, frontal eye field (FEF), LIP and sensory areas of the cortex, indirectly via ascending projections through the thalamus (Thompson and Bichot, 2005). A pathway through the mediodorsal nucleus to the frontal cortex (Sommer and Wurtz, 2008) transmits a corollary discharge of saccade-related activity arising in the intermediate SC. In contrast to the superficial layers, the deep layers

send efferents to thalamic nuclei that are not commonly considered to be visual processing structures (McHaffie et al., 2005), and to subthalamic and lower brainstem nuclei that are generally classed as motor areas or reticular formation (Harting et al., 1973; Benevento and Fallon, 1975; Graham, 1977), such that neurons in the deeper layers of the SC send an ipsilateral descending pathway to orienting motor systems, and a command signal is sent from the deeper layers of the SC to the saccade generator in the midbrain and pons (Sparks and Hartwich-Young, 1989; Harting. 1977, Baleydier and Magnin, 1979).

A descending contralateral pathway, the tectospinal tract, crosses at midbrain level and courses caudally terminating in various regions including regions of the pontine and medullary reticular formation, regions near and possibly within the abducens nucleus, the inferior olive, and cervical spinal cord (Harting et al., 1973; Harting, 1977; Castiglioni et al., 1978). This tract is important in the reflex of turning of the head in response to visual, auditory and somatosensory stimuli.

The SC is not only an important recipient of basal ganglia processed information but is also a critical source of input. Direct afferent connections target both the subthalamic nucleus and dopamine neurons in the ventral midbrain while indirect input to the striatum occurs via relays in the thalamus. In addition to a pathway through the thalamus to the basal ganglia, a direct projection from the deeper layers of the SC to the substantia nigra pars compacta has been validated in several species, including primates (Comoli et al., 2003; May et al., 2009; McHaffie et al., 2006). The substantia nigra pars compacta contains dopamine neurons which produce a prediction error signal, so have a key role in reinforcement learning (Niv and Schoenbaum, 2008; Schultz, 2010). Neurons in the substantia nigra pars compacta also respond to unexpected and salient sensory stimuli

(Redgrave et al. 2008; Schultz and Romo. 1990), which could be directed at least in part by inputs from the SC and related to shifts in spatial attention.

In light of this, the ascending outputs alert higher centres to changes in the functional conditions of deep-layer neurons. The commissural projections coordinate the activity of the two superior colliculi and may play a role in movements of the eyes in response to approaching targets (Edwards, 1977; Edwards and Henkel, 1978). The descending efferents are involved in initiating behavioural responses to stimuli by repositioning the eyes, head, limbs (Huerta and Harting, 1984b; Dean et al., 1986).

1.2.3. PROPERTIES OF COLLICULAR CELLS

Superficial layer neurons

There are six neuronal cell types in the superficial layers of the SC comprising of vertical narrow, vertical wide, horizontal, piriform, marginal, as well as satellite cells (Langer and Lund, 1974). Each cell type has a distinctive set of dendritic field characteristics, a regional distribution, and consistent axon characteristics. Superficial layer projection neurons comprise of the vertical cell subsets, whilst local interneurons encompass the piriform, stellate and horizontal cell types (Mize, 1992; Özen et al., 2000). It has been found that the visual receptive fields in the SC have inhibitory surrounds which enhance the precision of the representation of stimuli and location (Rizzolatti et al., 1974; Berman and Cynader, 1975).

Deep layer neurons

As with neurons in the superficial layers, those in the deeper layers also have a number of distinct morphologies (Norita, 1980), with synaptic terminals covering the somatic and dendritic surfaces of the larger of these neurons extensively (up to 83%; Behan et al., 1988).

Due to extensive variation in somatic and dendritic properties of these neurons, axonal distributions and firing properties have become an essential aspect of verifying morphological patterns and groupings within the deeper layers of the SC. At least five subclasses of neurons with distinct firing properties have been identified within InG. The largest population of InG neurons are those with a regular spiking firing pattern, a firing property thought to be critical in the generation of discrete motor commands to control precise movements (Helms et al., 2004).

Auditory neurons in the deep layers of the SC are relatively insensitive to pure tones, signalling the spatial location of sounds is a more crucial aspect of these neurons' responsiveness, preferring intricate sounds comprising of multiple frequencies (Horn and Hill, 1966; Wickelgren, 1971; Stein and Arigbede, 1972; Gordon, 1973; Graham, 1981). Unlike auditory neurons elsewhere in the nervous system, auditory-sensitive SC neurons habituate to repeatedly presented stimuli, and consequently are best suited for detecting novel sounds.

1.2.4. NEURONTRANSMISSION WITHIN THE SUPERIOR COLLICULUS

A variety of neurotransmitters including glutamate, GABA, acetylcholine, and monoamines exert modulatory control on the SC through various inputs, enabling sufficient sensory processing and behavioural responses by SC neurons. Glutamate has been demonstrated to convey visual information to the superficial layers of the SC via direct retinotectal fibres and cortical input (Binns, 1999; Kondo et al., 2000). Opticum neurons within the superficial layers of the SC receive direct retinal excitation mediated by both N-methyl-D-aspartate (NMDA) and non-NMDA receptors. Glutamate is also found in the tecto-tectal commissural connections, which contain excitatory fibres as well as inhibitory GABAergic fibres (Olivier et al., 2000).

The inhibitory actions of GABA have also been found to shape visual responses, with the SuG having some of the highest levels of GABA found in the CNS (Mize, 1992). At least three types of inhibitory interneurons (piriform, stellate and horizontal) are found in the SuG and several major inhibitory fibre tracts terminate within both the superficial and deep SC (Mize, 1992). Clark et al (2001) found that ionotropic $GABA_A$ and $GABA_C$ receptors display distinct distribution profiles in the superficial layers of the SC. $GABA_A$ receptors were located on the neuropil of most superficial layer neurons, with optic tract stimulation leading to $GABA_A$ receptor–mediated inhibition within the superficial layers of the SC, the main effect of which was to curtail the excitatory response to retinal inputs (Clark et al., 2001). By contrast, $GABA_C$ receptors tended to be located on the cell soma of a subset of neurons. $GABA_B$ receptors were labelled on patchy, presumably presynaptic, locations, and on neuronal cell bodies.

A projection from the retina to SuG containing monoamineoxidiase (MAO) has been found in the rat (Nakajima et al., 1996; 1998) and after enucleation, MAO terminals in SuG deteriorate. In hamsters, the double-labelling of catecholaminergic terminals in SuG with antibodies to tyrosine hydroxylase and dopamine-beta-hydroxylase suggests that the transmitter of this projection is more likely to be noradrenalin rather than dopamine (Arce et al., 1994). Both noradrenalin and dopamine are reported to have inhibitory effects on visual responses in SuG (Straschill and Perwien, 1971). However, responses to electrical stimulation of the optic chiasm are also diminished by 5-HT although the response to cortically evoked stimulation is unaffected. Therefore, it has been suggested that 5-HT gates retino-collicular input to the SuG via pre-synaptic receptors as a means of selectively enhancing the relative contribution of cortical input in SuG, at the expense of retino-collicular input, during periods of arousal (Mooney et al., 1996). A more detailed review of monoamine transmission is given in the relevant experimental chapters (Chapter 5 and Chapter 6).

Many sites for angiotensin I and II have been found in the SC, especially in the superficial layers (Bunnemann et al., 1992; Michels et al., 1994; Gehlert et al., 1991; Rowe et al., 1991). Local injections of angiotensin II into SuG suppress visually evoked potentials (Merabet et al., 1997; Marois et al., 1996) and reduce single neuronal responses to stimulation of the optic chiasm (Mooney et al., 1994), while antagonists of angiotensin I and II have the opposite effect. Given that the release of angiotensins into the blood is associated with behavioural states of motivation, attention and arousal (Kovacs and Dewied, 1994), this may provide a mechanism allowing these states to influence visual activity in the SC. Activity in the superficial SC has been shown to have some influence on the control of cardiovascular tone, changing blood pressure and heart rate (Keay and Redgrave, 1990). Also a local injection of angiotensin II (0.1±10 nM) into SuG has been shown to increase mean arterial blood pressure and cause bradycardia (DaMico et al., 1997). The nitric oxide synthetase inhibitor L-NAME also increases blood pressure when injected in to superficial SC (DaMico et al., 1998).

1.2.5. FUNCTIONAL PROPERTIES OF THE SUPERIOUR COLLICULUS

The SC has a wide variety of functions. It is a multimodal structure that integrates information about sensory saliency and attentional focus for the generation of goal-directed orientation movements towards novel sensory stimuli (Sparks and Nelson, 1987; Binns and Salt, 1997). Thus, the SC has a fundamental role in the analysis of novel situations to synthesize the optimal motor response immediately in the new situation encountered, which may be a critical decision in regards to survival.

Multimodality

Many neurons in the deeper layers of the SC show multisensory (visual, auditory, somatosensory) responses (Fecteau and Munoz, 2006). These sensory representations and motor neurons are well organised and form topographical maps of the external space and

body, which are in register with each other, and with the motor representations. Because most descending output neurons are the sites of extensive multisensory convergence, each of the sensory representations has access to at least some of the same efferent, or premotor circuitry. This enables the different sensory systems to initiate the same behaviours via some of the same neurons. Thus the SC provides a modality-independent, topographic representation of the locations of physically salient stimuli: valuable information for body, head and ocular orienting responses (Fecteau and Munoz, 2006; Winkowski and Knudsen, 2007).

Compared to the most efficient of the single modality-specific stimuli, cross-modal stimuli received from the same site on the topographic map produce multisensory interactions that significantly augment the response of a neuron (Wallace et al., 1996). However, stimuli derived from different locations, either produce no multisensory interaction, or an inhibitory effect; significantly depressing the response of the neuron (Wallace et al., 1996; Kadunce et al., 1997). Orientation responses are significantly enhanced when cross-modal cues are in spatial register, compared to those that are spatially disparate, suggesting physiological changes are paralleled by similar effects on SC-mediated overt behaviour (Stein et al., 1989). Different subclasses of output neurons have been shown to have different patterns and preferences in regards to modality convergences, at least in the rat, with somatosensory stimuli heavily influencing tecto-spinal neurons (Rhoades and DellaCroce, 1980; Chevalier et al., 1984; Westby et al., 1990; Keay et al., 1990), while those projecting to the contralateral pontine reticular formation are activated preferentially by auditory stimuli (Keay et al., 1990).

Interestingly, in the new-born monkey, Wallace et al. (1996) observed that SC neurons not only had increased response latencies, and approximately half the incidence of multisensory neurons when compared to adulthood, but the neurons did not yet integrate

the cross-modal inputs they receive. This suggests that the speeded gaze shifts to cross-modal stimuli is not possible at this age, as these behavioural responses are believed to depend on multisensory integration in SC neurons and the correct development of this system (Perrott et al., 1990; Hughes et al., 1994; Nozawa et al., 1994; Frens et al., 1995).

Saccade generation and orientation

Primates and humans are foveate animals and accordingly, detailed analysis of the visual scene requires the precise orienting of their visual axis, as to allow the high acuity fovea to resolve objects of interest. These saccadic eye movements are interspersed with intervals of active fixation during which the visual system executes a concise analysis of an object that may pertain to current goals. By moving the eye so that small parts of a scene can be sensed with greater resolution, body resources can be used more efficiently. Conversely, the rat is not a foveate animal and thus has a very wide visual field (de Araujo et al., 2001). Yet, the SC is implicated in the initiation of a broad spectrum of motor behaviours, including saccadic eye movements, fixation, the orientation of head, body and in some cases the ear towards novel sensory stimuli and approach and escape behaviours together with appropriate cardiovascular changes also occur through SC processing (Keay et al., 1988).

The integrity of the SC is crucial for the generation of saccades (Wurtz and Albano, 1980; Sparks and Hartwich-Young, 1989). The removal of the SC leads to sustained deficits in the generation of VGS (Schiller et al., 1980), Furthermore, it leads to the elimination of short-latency, extremely reflexive 'express' saccades (Fischer and Boch, 1983; Schiller et al., 1987; Isa, 2002). Additionally, the integrity of the SC is crucial for the cortical control of saccades by the FEF (Hanes and Wurtz, 2001), and is classed as a critical connection in the oculomotor networks (Hanes and Wurtz, 2001, see Figure 1.10 for the neural circuitry of the SC in controlling the planning and production of saccadic eye movements).

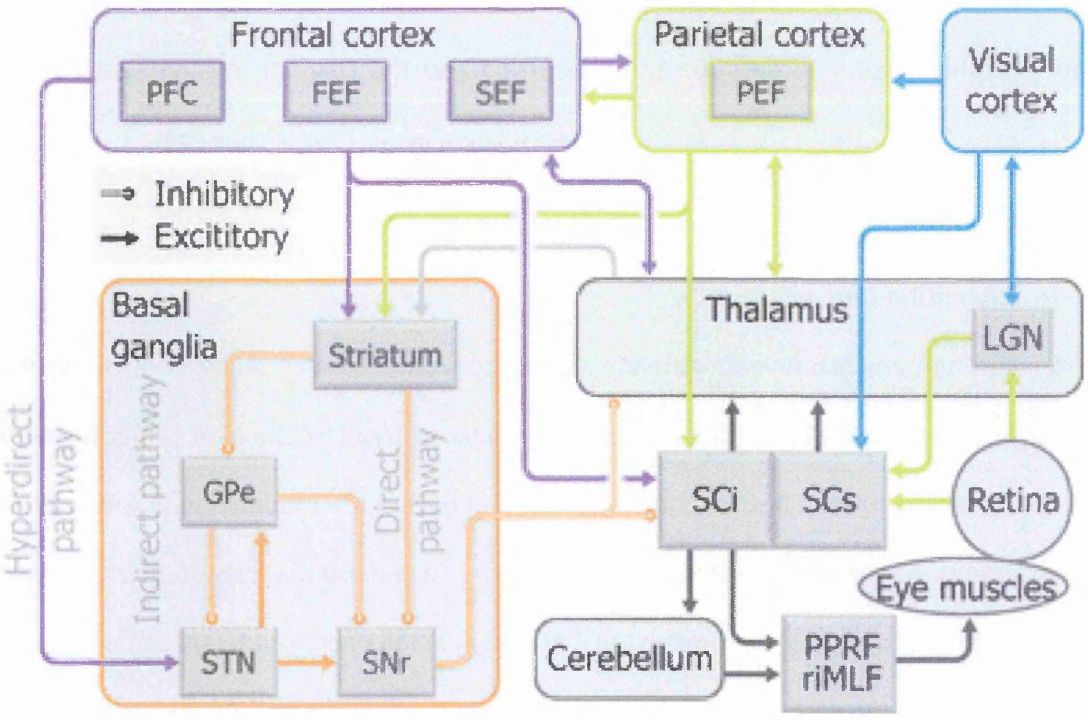

Figure 1.10: Neural circuitry of the SC in controlling the planning and production of saccadic eye movements. Many connections are not shown. FEF, frontal eye field; GPe, external segment of globus pallidus; LGN, lateral geniculate nucleus; PEF, parietal eye field; PFC, prefrontal cortex; PPRF, paramedian pontine reticular formation; riMLF, rostral interstitial nucleus of the medial longitudinal fasciculus; SCi, intermediate layers of superior colliculus; SCs, superficial layers of superior colliculus; SEF, supplementary eye field; SNr, substantia nigra pars reticulata; STN, subthalamic nucleus. Adapted from Douglas and Brian (2011).

It has been reported that patients with striate cortex (V1) lesions have the ability to localise visual targets in their scotoma with saccades without visual awareness (Poppel et al., 1973; Weiskrantz et al., 1974), a phenomenon known as 'blindsight'. In unilateral V1-lesioned monkeys, Kato et al. (2011) discovered that ipsilateral SC inactivation caused the monkey to lack this blindsight ability when the target was expressed at the injection site on the SC topographical map. This finding suggests that the retino-tectal pathway plays a role in residual (active) vision, and plays an important role in producing VGS. Spontaneous saccades, which are independent of visual inputs, towards the injection site were not terminated, thus, proposing the impairment of VGS subsequent to the inactivation of the

ipsilesional SC was not due to a saccadic motor deficit, but was primarily due to a visual deficit by conflicting with processing in the superficial layer.

Early studies of the primate SC first linked SC neuronal activity with the process of saccade target selection (Goldberg and Wurtz, 1972; Wurtz and Mohler, 1976). Studies demonstrated that neurons in the deeper layers of the SC exhibit high-frequency burst activity prior to spontaneous saccades in the dark (Goldberg and Wurtz, 1972). In monkeys, Robinson et al. (1991) also reported that upon electrical stimulation of the SC, associated saccadic eye movements were evoked, the magnitude and direction of which were dependent on the location of the stimulation. Similar results have been demonstrated in the SC in all classes of vertebrates (du Lac and Knudsen, 1990; Stein and Meredith, 1993). Similarly, neuronal activity in the SC is related to evaluating possible saccade targets, with SC neurons showing elevated activity for visual stimuli that will be selected as the end point of saccades, relative to those that are ignored (Glimcher and Sparks, 1992; Krauzlis and Dill, 2002; McPeek and Keller, 2002). For many neurons this modulation predicts the upcoming saccade, but for others it is related to the selected visual stimulus rather than to the movement itself (Horwitz et al., 2004; Horwitz and Newsome 1999; McPeek and Keller, 2002). Some aspect of SC activity is necessary for saccade target selection because chemically blocking activity disrupts saccade choices. When the target is placed in the affected part of the visual field, saccades tend to be erroneously directed to distracter stimuli located elsewhere (McPeek and Keller, 2004; Nummela and Krauzlis, 2010).

As previously mentioned, the motor-command neurons of the deep SC are like their fellow sensory neurons topographically organised, and the sharing of co-ordinates between the motor and sensory representations facilitates the direct translation of spatial cues into orientation responses (Sparks and Nelson, 1987). To this end, discrete regions of the motor representation project via different brain stem motor nuclei so that motor behaviours

originating from the SC are more highly dependent on the position from which sensory cues are emitted rather than the modality information they carry (Dean et al., 1988 ; 1989; Redgrave et al., 1990; Westby et al., 1990).

Stimuli salience map

The coding of visual saliency is critical for efficient neural processing (Itti and Koch, 2001). Early vertebrates, like their modern relatives, were faced with competing motivations and multiple sensory inputs, and thus, required a process to select the most salient stimuli and adaptive responses while suppressing less favoured alternatives. The frontoparietal network (DLPFC, FEF, LIP, sensory areas of the cortex, and the nuclei in the thalamus that interconnect these structures), is involved in enhancing the processing of some visual stimuli, allowing them to be more salient than others (Thompson and Bichot, 2005; Bisley, 2010; Knudsen, 2011). As the primary structure responsible for re-directing gaze toward or away from unexpected novel events (Dean et al., 1989; Stein and Meredith, 1993), recent evidence has suggested the SC also plays an important role in target selection based on saliency (Kundsen, 2011; Shen et al., 2011). Notably, the SC interconnects extensively and can manipulate responses within the frontoparietal network. The retino-tectal visual system can simultaneously represent numerous events, each one of which could potentially initiate a change of gaze. A selection architecture that can evaluate which of multiple simultaneously presenting stimuli is the most urgent, is essential. If the superior colliculus is to be able to achieve accurate orientation responses, the sensory neurons must be able to determine which stimuli in the environment are novel and provide precise information about their location. In mammals, the superficial layers of the SC send a high spatial-resolution, retinotopic depiction of the sites of salient visual stimuli to the forebrain via the thalamus nuclei, LGN and PULi (Reiner and Karten, 1982; Boehnke and Munoz, 2008). Thus, the superficial SC, through this pathway, has the functional ability to decrease thresholds and augment response gains and resolution in the retinotopically organised visual

forebrain areas (Luck et al., 1997; Reynolds and Chelazzi, 2004; Shipp, 2004; Maunsell and Treue, 2006). Via the dorsal pulvinar (PULd), and anterior thalamus, the deeper layers of the SC transmit lower resolution (larger receptive fields), multimodal information to the parietal and prefrontal forebrain regions (Shipp, 2004; Kaas and Lyon, 2007; Boehnke and Munoz, 2008), thus, closing feedback loops with the FEF and the LIP (Thompson and Bichot, 2005; Fecteau and Munoz, 2006; Bisley and Goldberg, 2010; Falkner et al., 2010). It is important to consider the ability of the SC to override and manipulate this frontoparietal network when choosing visually salient stimuli as the next focus of gaze and attention.

It has been suggested that, the appropriate selection of stimuli does not occur locally in the SC's sensorimotor maps (Snaith and Holland, 1990). A key system to execute the pre-attentive selections involved in a gaze shift, including which stimulus is salient, is thought to be the looped architecture connecting the SC to the basal ganglia via the dorsal thalamus (McHaffie et al., 2005). Note that a pause in inhibitory nigrotectal output activity has been found to precede the initiation of gaze shifts to unpredicted sensory events (Hikosaka et al., 2000).

In the monkey, enhanced and sustained responses occur in SC neurons if a selected visual stimulus as the target for a shift in gaze (Wurtz and Mohler, 1976; Li and Basso, 2005, 2008), perceptual judgement, or as a goal for future action (Wurtz and Albano, 1980; McPeek and Keller, 2002; Horwitz et al., 2004), is found in the neuron's receptive field. McPeek and Keller (2004) found that the probability a monkey will choose an oddball stimulus (based on colour or luminance) from a group of similarly salient stimuli as the target for a shift in gaze was greatly reduced when the oddball stimulus was represented in an inactivated portion of the SC space map. In contrast, presentation of the oddball stimulus alone had no impairment on selection. Therefore, this suggests that the role of the SC in

stimulus selection is vital when a monkey is required to select among uniformly salient, competing stimuli (McPeek and Keller, 2004; Lovejoy and Krauzlis, 2010).

Attention

As previously mentioned, the SC is part of an integrated circuit for processing of spatial sensory information and orienting responses, and is a key part of the posterior attentional system (see Section 1.1.4). It is part of a network that functions in directing saccadic eye movements, overtly shifting both gaze and attention in space, thus controlling spatial attention and target selection.

Extensive interactions between the deep layers of the SC with the DLPFC, LIP and FEF, in the forebrain network, seem to be essential for sustaining spatial attention at a specific location (see Figure 1.11). Forebrain structures such as the DLPFC, LIP, FEF and visual cortex convey information about the behavioural relevance of a stimulus to the SC (Sommer and Wurtz, 2000; Thompson and Bichot, 2005; Johnston and Everling, 2006; Bisley and Goldberg, 2010). So, by integrating this information with the assessment of the physical salience of stimuli, a retinotopic depiction of the relative importance of locations as the subsequent locus for the orientation of attention and gaze is produced by the circuitry in the SC (Fecteau and Munoz, 2006; Dorris et al., 2007; Shen and Pare, 2007; Mysore et al., 2011). Evidence of this includes the findings that SC electrical stimulations cause a shift in a monkey's attention to the location corresponding to the stimulation site (Muller et al., 2005), and monkeys lose the ability to sustain attention at a position in space when the SC, LIP or FEF is inactivated (Wardak et al., 2004, 2006; Lovejoy and Krauzlis, 2010). Rats with SC-lesions were deficient in problem solving ability which needed a correct function of orienting behaviour and attention (Weldon and Smith, 1979; Midgley and Tees, 1986), emphasising the importance of the SC interaction with other neural systems in processes

mediating the direction of attention. Interestingly, the SC-lesioned animals are also hyperactive; another symptom of ADHD (Weldon and Smith, 1979). The SC has the capacity to override top-down influences by selecting and immediately initiating a shift of gaze and attention toward a highly salient distracting stimulus (of any sensory modality) due to its novelty, motion or intensity as the next focus of attention (Wardak et al., 2004, 2006; Lovejoy and Krauzlis, 2010). The capacity to over-ride now invalid prepotent automatic behaviours in accordance to novel situational demand is crucial for all mammals (Fernandez-Dugue et al., 2000).

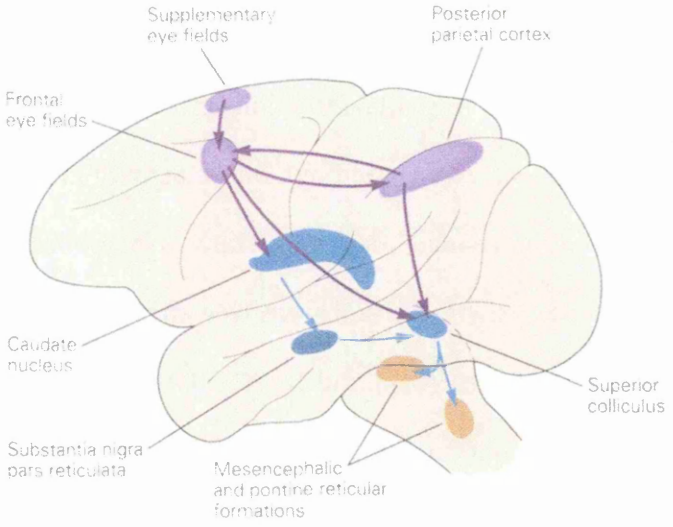

Figure 1.11: Extensive connections between the deeper layers of the SC with the dorsolateral prefrontal cortex, lateral intraparietal cortex and FEF, in the forebrain network, seem to be essential for sustaining spatial attention at a specific location in primates. Adapted from Goldberg and Buhnell (1981).

The SC is also a crucial component in the control of covert spatial attention, a process that focuses attention on a region of space different from the point of gaze (Goldberg and Wurtz, 1972; Ignashchenkova et al., 2004). Following stimulation of the SC, in the corresponding area of the visual field in the monkey, psychophysical performance improved without the production of saccades (Muller et al., 2005). The spatial selectivity of the effect (Muller et al., 2005) strongly suggests that the SC activity functions in the control of covert

visuo-spatial attention, and that the effect was not due to indiscriminate arousal or vigilance.

The attentional effects caused by SC stimulation are due to its capability of activating and manipulating a larger network of areas that together control attention and may determine the next locus of attention. The disconnection of the SC from the prefrontal cortex controlling influences leads to an increase in distractibility as has been shown in humans (Gaymard et al., 2003). A decrease in distractibility has also been observed in SC-lesioned animals in an array of species (cat: Sprague and Meikle, 1965; rat: Goodale et al. ,1978; monkey: Milner et al., 1978). Lovejoy and Krauzlis (2010) found that SC inactivation caused large and spatially specific deficits in spatial attention yet without signs of motor deficit. In light of this, a recent experiment was carried out where activity in the SC was chemically inactivated during a motion-change detection task while neuronal activity was simultaneously recorded in two cortical visual areas well known to be modulated by spatial attention: the medial temporal area (MT) and the medial superior temporal area (MST) (Zénon and Krauzlis 2012). Zénon and Krauzlis (2012) found no changes in attention-related effects in visual cortex, such as spatial attention, or changes in the neuron's ability to detect stimuli in these two cortical visual areas despite the presence of large deficits in attention. This suggests that the spatial attention deficits seen by Lovejoy and Krauzlis (2010) and Zénon and Krauzlis (2012) were not due to changes in the ascending SC to visual cortex pathway and therefore sub-cortical attentional pathways must play a vital role in these attentional deficits.

The role of the SC in orienting gaze towards stimuli in the environment is well established in all classes of vertebrates (Stein and Meredith, 1993). Given the conservative nature of the evolution of neural circuits (Katz and Harris-Warrick, 1999), including the SC (Overton,

2008) it is improbable that functions critically dependent on the SC are entirely replaced by cortical areas in primates. Importantly, all vertebrate species must have the capacity to select information for processing based on its relevance to behaviour. For example the SC is still a central structure in the mediation of visual behaviour in catarrhine primates, such as old world monkeys (Shen et al., 2011) and the function of the SC in selecting stimuli for attention has been demonstrated in monkey (McPeek and Keller, 2004; Lovejoy and Krauzlis, 2010). Functional similarities such as deficits in sensory attention and orientation have also been reported in SC-lesioned rats (Weldon and Smith, 1979; Midgley and Tees, 1986). In spite of dissimilarities in aspects of the SC and connections that reflect diversity in the cognitive capabilities and vast evolutionary divergence of species, the preservation of the SC throughout evolution suggests it embodies fundamental circuits and mechanisms for competitive stimulus selection and attentional focus in all vertebrates, including humans.

1.2.6. EFFECTS OF ADHD MEDICATION ON THE SUPERIOR COLLICULUS

The mechanism of action of the drugs used in the treatment of ADHD such as amphetamine and methylphenidate is not fully established but one possibility is that the drugs affect the SC. There is evidence for this from research on saccades. For example, in a MGS task, Mostofsky et al. (2001) found un-medicated children with ADHD showed longer saccade latency, while the methylphenidate medicated group had a drastic improvement in saccade latency which was comparable to that of the age-matched control. Moreover, Chee (1991) observed spontaneous emissions of high-voltage alpha wave electrophysiological activity in the SC and, the incidence of this activity was suppressed by increasing doses of D-amphetamine. Also, Easton et al. (2007a) found that the pharmacological magnetic resonance imaging blood-oxygenation-level-dependent (BOLD) response was augmented in the rat SC following administration of D- and L-amphetamine sulphate isomers. This could

suggest that ADHD pharmacotherapies affect brain regions, such as the SC, and this underlies the therapeutic effects in ADHD patients.

As previously mentioned, an increase in synaptic availability of the monoamines dopamine, noradrenalin (Azzaro and Rutledge, 1973; Easton et al., 2007b) and serotonin (Holmes and Rutledge, 1976; Kuczenski and Segal, 1989) mediates the acute effect of amphetamine administration. Expansive serotonergic innervation, preferentially innervating the superficial layers is found in the SC (Parent et al., 1981; Weller et al., 1987), while restricted noradrenergic (Lindvall and Bjorklund, 1974; Weller et al., 1987) and dopaminergic input (Weller et al., 1987; Campbell et al., 1991) have been reported. This suggests that serotonin is the dominant monoamine affected by amphetamines in the superficial layers of the SC.

Gowan et al. (2008) found the administration of D-amphetamine produced dose-dependent depression of the amplitude and duration of responses to whole field light flash stimuli in the superficial layers of the SC. At the highest doses of D-amphetamine administered, visual responses were entirely suppressed. However, in the cat, D-amphetamine augmented responses in the superficial layers of the SC when a stimulus was displayed within the excitatory centre of the cells' receptive fields only (Grasse et al., 1993). It is possible that D-amphetamine amplifies the signal-to-noise ratio as it suppresses responses to stimuli which give relatively minimal levels of SC activation (as in the sub-optimal whole field light stimuli; Gowan et al., 2008), and augments responses to stimuli which give relatively high levels of activation (such as stimuli limited to the excitatory centre; Grasse et al., 1993).

In support of this theory, Dommett et al. (2009) found that therapeutically relevant doses of D-amphetamine and methylphenidate increased the signal-to-noise ratio in the SC by suppressing weak and preserving strong activations mediated by serotonin via a pre-

synaptic mechanism. It has been shown that serotonin reduces the signal-to-noise ratio in the somatosensory cortex (Waterhouse et al., 1986) and thalamus (Funke and Eysel, 1993), yet not by altering the weak and strong signal relationship but by suppressing spontaneous background activity. Furthermore, intra-collicular micro-injection of a serotonin$_{1B\text{-}1D}$ receptor agonist reduces distraction in behaving animals (Boulenguez et al., 1995). The behavioural effects of these drugs could be linked to a change in the signal-to-noise ratio effect via actions on the SC, biasing the system towards salient stimuli and consequently leading to a reduction in distractibility.

It has been hypothesised that the SC could 'bid' for motor expression. Thus, heightened activity can be thought as placing a stronger "bid" into the central selection device thought to be the basal ganglia (Chevalier and Deniau, 1990), increasing the likelihood of saccade generation. By efficiently decreasing the response, or 'bid' for weak stimuli, psychostimulants have the ability to bias the system so that distractions only arise to very salient stimuli. This would therefore cause a reduction in overall distractibility and an enhancement in sustained attention, as seen in people without ADHD, and ADHD sufferers following psychostimulant administration. Critically, if the SC is dysfunctional in ADHD, this may signify the SC as a crucial novel target for the development of non-addictive pharmacotherapies for ADHD. Therefore by examining the function of the SC in an ADHD model rat and suitable control strains, it may be possibly to shed further light on the underlying neuropathology in ADHD and learn more about the validity of the model.

1.3.ANIMAL MODELS OF ADHD

An ideal animal model should be similar to the disorder it models in terms of etiology, physiology, symptomatology, and treatment effects, and should ultimately make predictions about future therapies. Thus, an ADHD animal model must mimic the fundamental behavioural characteristics of ADHD (face validity). It should also conform to an established

or hypothesised pathophysiological basis of the disorder (construct validity). Not knowing the exact basis of ADHD is problematic for the development of animal models and assessing construct validity of existing models, however, it is assumed that some deficits would need to be found within the monoamine systems in the very least in order to demonstrate construct validity. Finally, any animal model of ADHD ought to predict features of the disorder as well as treatment efficacy (predictive validity) (Sagvolden, 2000; Sagvolden et al., 2005).

1.3.1. ADHD ANIMAL MODEL OVERVIEW

Several animal models of ADHD have been proposed that have been developed using three main strategies (see Table 1.2). The first strategy is to select animals that exhibit the core behavioural characteristics or specific components. The second is to simulate the postulated pathology by making lesions. The third strategy is to use genetic manipulation of candidate genes to produce transgenic animal models deriving from etiological hypotheses.

Animal Model	Modification Strategy	Face Validity	Predictive Validity	Construct Validity	Problems with the model
SHR	Bred for hypertension	Attention/ learning deficits, hyperactivity, impulsivity	Some symptoms reduced by monoaminergic agents	Dysfunctional fronto-striatal system	Hypertension, WKY rats as a control group
GH	Bred for hypertension	Impulsivity	No data on predictive validity	Dysfunctional fronto-striatal system	Hypertension, Lacks hyperactivity
DAT-KO	Knock-out of DAT gene	Hyperactivity, spatial memory deficits	Hyperactivity reduced by psychostimulants	Alterations to the DA system	No hints for reduced DAT in ADHD
Dopamine-depleted animals	Destruction of DA neurons	Hyperactivity	Hyperactivity reduced by psychostimulants	Alterations to the DA system	Lacks attentional deficits and impulsivity
Coloboma mouse	Mutation on SNAP-25 gene	Hyperactivity, impulsivity	-	Alterations to the DA and NA system	No data on predictive validity Role of SNAP-25 in ADHD unclear
Naples high-excitability rat	Bred for excitability	Hyperactivity	-	Alterations to the DA system	No data on predictive validity or impulsivity
Accallosal mouse	Agenesis of the corpus callosum	Hyperactivity, learning difficulties	-	Reduced callosal regions found in patients with ADHD	Role of corpus callosum in ADHD unclear No data on DA or NA systems No data on predictive validity

Table 1.2: The main animal models of ADHD. SHR: spontaneous hypertensive rat; GH: New Zealand genetically hypertensive rat; WKY: wistar Kyoto; DAT- KO: dopamine transporter knockout; DA: dopamine; SNAP-25: Synaptosomal-associated protein 25; NA: noradrenalin. Adapted from Sontag et al. (2010).

New Zealand genetically hypertensive rat

The genetically hypertensive rat (GH), was developed in New Zealand by selective breeding of Wistar rats for hypertension (Smirk and Hall, 1958; Phelan, 1968; Simpson et al., 1973). The GH show some face validity, with core behaviours such as impulsivity (Wickens et al., 2004) and delay of reinforcement deficits (Sutherland et al., 2009). Yet, the GH show no evidence of hyperactivity within an open field in comparison with its parent strain, the Wistar (McCarty and Kopin, 1979; McCarty and Kirby, 1982; McCarty, 1983). The GH is a

promising animal model of ADHD, yet it loses some face validity with the lack of hyperactivity. Similarly further work is needed to analyse the behavioural characteristics of the GH, for example the effects of methylphenidate have not been tested on these animals, and little construct validity has been established.

Dopamine-depleted animals

The experimental destruction of DA-containing neurons with 6-hydroxydopamine (6-OHDA) in adult rats is an established model of Parkinson's disease. There are no attentional deficits or impulsivity seen in these animals, yet lesions of the dopaminergic system in neonatal rats lead to age-limited spontaneous motor hyperactivity (Creese and Iversen, 1973; Heffner and Seiden, 1982; Luthman et al., 1989, 1997; Shaywitz et al., 1976a, b) that can be normalised by psychostimulants (Davids et al., 2002; Heffner and Seiden, 1982; Luthman et al., 1989; Shaywitz et al., 1976a). These deficits disappear in adult rats, probably due to ongoing developmental processes. The model has some construct validity as clearly the dopaminergic system is affected. Data suggest that increased D4 receptor levels in the caudate-putamen correlate with behavioural hyperactivity (Zhang et al., 2001). Furthermore, the D4 receptor seems to be essential for hyperactive behaviour (Avale et al., 2004a) as mice with neonatal 6-OHDA lesions lacking the D4 receptor did not show hyperactive behaviour compared to the wild type (Avale et al., 2004a). In neonates, the effects of the lesions vary according to specific details of the protocols, such as the age of the rat at treatment, the dose, the age of animal at testing and the degree of subsequent hyperactivity is correlated with the extent of the dopamine depletion (Miller et al., 1981). However, the hyperactivity in this model is not necessarily a primary effect of low dopamine levels but possibly a secondary effect due to compensatory overgrowth of another neurochemical pathway, such as serotonin. For example, a serotonergic hyperinnervation of the striatum was found following 6-OHDA lesion (Descarries et al., 1992; Frohna et al., 1997; Kostrzewa et al., 1998; Luthman et al., 1990; Stachowiak et al.,

1984; Towle et al., 1989; Zhang et al., 2002a). A study by Avale et al. (2004b) suggests that this increase in striatal serotonin is associated with hyperactive behaviour. Avale and colleagues treated mice with neonatal 6-OHDA lesions with a tryptophan hydroxylase inhibitor in order to normalise striatal serotonin without affecting dopamine levels. These mice did not show hyperactive behaviour. In summary, this model shows some predictive validity, since treatment with psychostimulants reduces the hyperactivity. Construct validity is given by the profound changes in the catecholaminergic neurotransmitter system. Finally, the hyperactivity of this model supports face validity, but the model has not been validated using tasks that measure behavioural characteristics such as impulsivity or specific attentional deficits and therefore at present the face validity is limited. However, this model enables the study of the role of the D4 receptor and serotonin in ADHD.

Dopamine transporter (DAT) knockout mouse

The DA transporter knockout (DAT-KO) mouse lacks the dopamine transporter (DAT) gene and shows some face validity as it has ADHD-like symptoms such as spontaneous behavioural hyperactivity (Gainetdinov et al., 1999; Gainetdinov and Caron, 2001; Giros et al., 1996). However, like the dopamine depleted animals described above, face validity is limited to hyperactivity because the DAT-KO mouse does not exhibit attentional deficits or impulsivity. The hyperactivity observed in DAT-KO mice is associated with a marked decrease in dopamine clearance (Jones et al., 1998a), which is most likely due to the lack of the DAT. This lack has been shown to induce several compensatory changes such as a decrease in dopamine release from nerve terminals (Gainetdinov et al., 1998; Jones et al., 1998a) causing a significantly lower extracellular dopamine concentration. The alteration in dopamine functioning therefore gives this model construct validity. This animal model also shows predictive validity as hyperactivity is normalised by psychostimulant treatment (Gainetdinov et al., 1998; Gainetdinov and Caron, 2001; Jones et al., 1998b). Despite the primary actions of amphetamine and methylphenidate being on the dopamine transporter

– these drugs still reduce hyperactivity in this knockout model. This is likely to be due to alterations of the noradrenergic system rather than the dopaminergic system.

Coloboma mouse

The coloboma mutant mouse was developed using neutron irradiation (Searle, 1966). This mouse shows delayed neurodevelopment and behavioural deficits such as motor hyperactivity, impulsivity and impaired inhibition in a delayed reinforcement task (Bruno et al., 2007; Hess et al., 1994, 1996; Heyser et al., 1995; Wilson, 2000). The hyperactivity observed can be reduced by D-amphetamine but not by methylphenidate (Hess et al., 1996; Wilson, 2000). Since this mouse has a mutation on the SNAP-25 gene, it is likely that the behavioural deficits are related to a SNAP-25 dysfunction (Hess et al., 1992; 1996; Steffensen et al., 1996). The SNAP-25 protein is essential for the fusion of the neurotransmitter vesicle with the presynaptic membrane in order to release neurotransmitters. This might explain why the dopamine release in the dorsal striatum of the coloboma mutant mouse is almost completely lost (Raber et al., 1997). In addition, the D_2 receptor expression is increased in the ventral tegmental area and substantia nigra compacta (Jones et al., 2001b). Alterations in the noradrenergic system such as an increased noradrenalin concentration in the striatum, LC and nucleus accumbens were also observed (Jones et al., 2001a). NA depletion following the administration of the neurotoxin N-(2-chloroethyl)-N-ethyl-2-bromobenzylamine (DSP4) has been shown to reduce the hyperactivity but not the impulsivity (Bruno et al., 2007; Jones and Hess, 2003). The alterations of the catecholaminergic systems support the construct validity of this mouse. Face validity is given by the behavioural deficits, and predictive validity is given through the effects of amphetamine. However, the role of the SNAP-25 gene in ADHD remains to be investigated, similarly SNAP-25 gene alterations must have a global effect on the brain, that therefore cannot only be linked to dopamine.

Naples high excitability rat

Naples high-excitability rats are selected based on increased exploration behaviour, although this is dependent on the environment, with these animals not hyperactive in a familiar environment (Sadile, 1993). This is unlike children with ADHD, who do exhibit hyperactivity in familiar environments. However, this model does show deficits in visual-spatial attention (Aspide et al., 1998; Gallo et al., 2002; Papa et al., 2000). Face validity of this model is therefore supported by the presence of some motor hyperactivity and attentional deficits, but it shows no impulsivity. Further investigations have shown that these rats have alterations in the dopaminergic system. For example, tyrosine hydroxylase, DAT and D2 receptor mRNA are hyperexpressed in the PFC, while the D1 receptor is down-regulated (Viggiano et al. 2002a; 2000b; 2003a; 2003b; Viggiano and Sadile, 2000). Construct validity is given because these deficits are probably based on altered dopaminergic function in the forebrain. However, studies regarding the effects of psychostimulants on the deficits observed are still lacking, and therefore the model has no predictive validity.

Acallosal mouse

Acallosal mice have a complete agenesis of the corpus callosum. The model has slight face validity as there are signs of hyperactivity, such as a reduced number of brief stops and a decrease in habituation in an open field (Magara et al., 2000), yet there is no information on the impulsivity and attentional deficits in these animals. The model has some construct validity as reduced sizes of callosal regions have been found in some patients with ADHD (Baumgardner et al., 1996; Giedd et al., 1994; Hynd et al., 1991; Semrud- Clikeman et al., 1994). As possible alterations in the monoaminergic system and the effects of psychostimulants are still lacking, the model has relatively weak validity.

1.3.2. THE SPONTANEOUSLY HYPERTENSIVE RAT

All the models described above have not been widely used because of the limitations in validity. The main animal model used in ADHD research is the spontaneously hypertensive rat (SHR). In Japan in the early 1960s, the SHR was produced by inbreeding the Wistar-Kyoto (WKY) rat strain (Okamoto and Aoki, 1963). During the inbreeding of the SHR for the high blood pressure trait, unexpectedly the selection also produced increased activity (Qian et al., 2010), motor and cognitive impulsivity (Pardey et al., 2009), deficits in sustained attention (Jentsch, 2005) and alterations in monoamine transmission (Russell, 2003), all characteristics of individuals with ADHD. Subsequently, the SHR has become the most widely used animal model for ADHD and as such a further review of this model is provided below.

1.3.2.1. FACE VALIDITY

Attention

A deficit of sustained attention is evident in individuals with ADHD (Conners et al., 1996), and a variety of behavioural studies have found SHRs to be inattentive in comparison to the WKY (Berger and Sagvolden, 1998; De Bruin et al., 2003; Jentsch et al., 2005; Li et al., 2007), thus, providing support for the face validity of these animals as an animal for ADHD. Li et al. (2007) compared juvenile male SHR, with age- and gender-matched WKY, and found SHRs to be less attentive, but without memory deficits in the water maze task using a measure of the time it takes for the animal to find the opaque platform from memory. Using a lateralised reaction time task, Jentsch et al. (2005) found a pattern of behavioural changes that suggest a visuospatial divided attention deficit in the SHR compared to the WKY. A lateralised reaction time task involves the animal being placed in an operant chamber with two nose poke apertures. The animal responds by a sustained nose poke following a light target presentation over the correct nose poke hole. This results in a pellet being delivered, and a 'correct' choice is scored. Compared with the control group, SHR not only made fewer

correct choices and more incorrect choices but had difficulties in stimulus detection and processing when target durations were briefer than 1.0 s. Therefore, these data support the conclusion that SHR exhibited a fairly substantial and robust attention deficit (Jentsch et el., 2005).

De Bruin et al. (2003) also found a similar impaired attentional performance in the SHR using a five-choice serial reaction time task. The 5-choice serial reaction time test (5-CSRTT) originated from the continuous performance test (CPT) in humans. This task uses a 5-hole apparatus operant chamber, where rats are required to discriminate spatially a short visual stimulus occurring randomly in one of five locations. During the testing period, a rat is required to allocate its attention sufficiently that it is able to discriminate the location of the brief stimulus and maintain a sufficient activity level so that it can respond appropriately. Response accuracy, a marker of attention in the 5-CSRTT, was reduced in SHR in comparison to WKY and Sprague Dawley (SD) rats. The findings from these studies indicate the deficit in selective visual attentional performance found in the SHR strain is a robust phenotype.

Even though behavioural tests such as the 5-CSRTT and tests assessing vigilance decrement are available, few studies using these tests have been performed with SHR. Instead, to assess sustained attention in the model, extinction paradigms have been commonly used by many researchers to compare SHR and WKY (Berger and Sagvolden, 1998; Sagvolden et al., 1998; Sagvolden et al., 2005; Pardey et al., 2009). Interestingly, in the absence of a reinforcer, Pardey et al. (2009) found SHRs did not persistently respond at a significantly higher rate than the WKY, as observed by Berger and Sagvolden (1998), thus suggesting the SHR do not have a deficit of sustained attention in comparison to the WKY strain. Among the differences between the protocols of Pardey et al. (2009) and Berger and Sagvolden (1998), it has been suggested that the use of male rats by Pardey et al. (2009) may explain

the difference in results. Berger and Sagvolden (1998) found inattentive deficits to be more pronounced in the female SHR when compared to male SHR and the WKY control. This may suggest further face validity as the predominantly inattentive presentation of ADHD is most prevalent in human female suffers (Taylor et al., 1998). However, it is important to note that the validity of the extinction paradigm as an assessment of attention is questionable (McGaughy and Sarter 1995).

A difference in baseline response rates between SHR and WKY has been suggested to affect the results and conclusions of studies regarding the attentional deficit in the SHR (Alsop, 2007). Alsop (2007) assessed the results of extinction studies that imply a deficit of sustained attentional performance in the SHR, and found the results were due to the significant continuation of higher rate responding in comparison to the WKY (Sagvolden et al., 1993; Boix et al., 1998). Alsop (2007) found that by plotting the rates as a proportion of the maximum response rate during the task for each group, the differences between groups were greatly reduced; SHR and WKY actually displayed similar behaviours on these tasks. This suggests that SHR and WKY do behave in a similar way, but the overall level of behaviour was greater for the SHR due to the activity differences between the strains. This is not the case observed in children with ADHD in comparison to controls (Sagvolden et al., 1998).

Hyperactivity

Hyperactivity in the rat has been tested by activity levels in open field experiments and response rates in free-operant tasks. In various behavioural studies (Whitehom et al., 1983; Sagvolden et al., 1992; Li et al., 2007; Pardey et al., 2009; Qian et al., 2010) juvenile SHR have been shown to have an increased locomotor activity in comparison to WKY, their progenitor controls. The hyperactivity of these animals is seen in the juvenile (4-6 week old) stage of their development, prior to the development of hypertension (Sagvolden et al.,

2005). The absence of hyperactivity in the animals upon adulthood is also further support for the face validity of the model, as even though ADHD is still present in a high percentage of adult humans diagnosed in childhood, the hyperactive symptoms often diminish (Adler and Chua, 2002).

The majority of behavioural studies assessing hyperactivity in rodents use the open field test. The open field test is an open area that allows the rodent to move freely (it may have surrounding walls to prevent escape). Commonly, the field is marked in a square grid network. A high level of exploratory behaviour is typically displayed in rats exposed to a novel environment (Qian et al., 2010). As the novel environment becomes more familiar, such as when the rats are repeatedly placed into the same open field, or following a prolonged exposure to an open field within a session, a progressive reduction in exploratory behaviour occurs. This is known as the habituation profile. In the open field test, SHR, in comparison to the WKY, exhibit a continuous hyperactivity within the habituation phase (where most animals would have habituated to the environment), despite not showing hyperactivity when initially placed in the novel environment (Li et al., 2007; Pardey et al., 2009). Li et al., (2007) also found similar hyperactivity in the Morris water maze in the SHR strain compared to WKY. The Morris water maze consists of a submerged opaque platform placed somewhere within a pool of water (Morris et al., 1982). When placed in this pool, rats try to find a way out, they initially swim randomly until they find the platform and climb out. Healthy rats quickly learn the location of the platform and if the platform is removed, the rats search at the place where the platform had been (Morris et al., 1982). It can be used to measure hyperactivity by measuring the amount of swimming activity. These findings support the face validity of the SHR as the model of ADHD as the hyperactivity behaviour observed is similar to what has been stated in children with ADHD (Porrino et al., 1983, Sagvolden et al., 2005) who display locomotor hyperactivity in a familiar, but not in a novel environment and diminishes with age.

The data for the open field test is not always consistent, as sometimes the control strains are more active than the SHR (Sagvolden et al., 1993; Ferguson et al., 2003). Van den Bergh et al. (2006) found SHR to be more active in the open field than WKY, but only at specific ages. The use of the WKY strain, a well-known animal model for depression (Yamada et al., 2011), as a control in hyperactivity studies has been questioned, especially with analysis of the Morris water maze task, as this is also a task used to measure depression-like behaviour (Sun and Alkon, 2002). The WKY strain has several behavioural abnormalities itself, such as hypoactivity and a depression-like phenotype (Yamada et al., 2011). To overcome the problems with the use of WKY, Qian et al. (2010) evaluated the exploratory activity and habituation profile of SHR to that of a more active strain, the Wistar rat (WIS). Qian et al. (2010) observed both SHR and WIS displaying similar locomotor activity during the initial exploratory phase of open field exposure, signifying that the increased locomotor activity in SHR was not generated by the environment being novel. Instead, the hyperactivity of SHR was found during the habituation phase, similar to the results using WKY as a control.

Impulsivity

The SHR displays impulsive behaviour that has several features in common with ADHD behaviour characteristics. An abnormal response to reward in the SHR has been described. Impulsivity in the SHR has been extensively studied on a compound schedule of reinforcement that includes a fixed interval (FI) component followed by an extinction (EXT) component (Sagvolden et al., 1992a; 1992b; 1993a). Both SHR and control rats on this FI-EXT schedule show the typical 'FI scallop', which is an increase in response rate over the later segments of the FI component (see Figure 1.12). However, the rate increase is greater in the SHR than in control strains, with a greater terminal response rate (Sagvolden et al., 1992a; 1993b). In line with the theory that ADHD can be linked to altered reinforcement processes, there is a strong indication that the SHR may have altered reinforcement processes (Sagvolden et al., 1998) comparable to the possible origin of behavioural

disturbances in children with ADHD (Sonuga-Barke et al., 1992). Similarly, in the Delayed Reinforcement (DR) task, rats are trained to respond with a nose-poke to one of two visual stimuli; one response resulted in a small quantity of reinforcer, the other in a larger quantity of reinforcer. As the session proceeds an increasing delay is introduced onto the response leading to the large reward. Therefore, the nature of the choice is whether to have a small quantity of reinforcer immediately or a larger but progressively delayed amount of reinforcer. The SHR has been shown to choose the small quantity of reinforcer immediately, as the delayed reinforce increases, at a lot faster rate than the control strains (Sagvolden et al., 1992b). The pattern of responses on the Delayed Reinforcement ask and FI-EXT schedules seen in the SHR is consistent with the steepened delay-of-reinforcement gradient observed in individuals with ADHD (Sagvolden et al., 1998), suggesting strong face validity in this behaviour. Other research has also described a steepened delay-of-reinforcement gradient in SHR compared to controls by utilising different associated behavioural tasks (Johansen and Sagvolden, 2005; Johansen et al., 2005; Johansen et al., 2007). The SHR also show abnormal responses to reward (Wultz et al., 1990; Hendley and Ohlsson, 1991; Wultz and Sagvolden, 1992; Sagvolden et al., 1993b), which are similar, in several respects, to the altered reward sensitivity seen in children with ADHD. Like children with ADHD (Sagvolden et al., 1998; Castellanos and Tannock, 2002; Johansen et al., 2002), behaviour in the SHR is said to be more sensitive to immediate reinforcement and proportionately less sensitive to delayed reinforcement (Sagvolden et al., 1992b). Also, like children with ADHD, more frequent reinforcement reduces the differences between the SHR and controls (Sagvolden et al., 1993a). In direct measures of the effect of delay of reinforcement, SHR are more impulsive than the WKY as defined by preference for smaller, immediate reinforcers over larger, delayed ones.

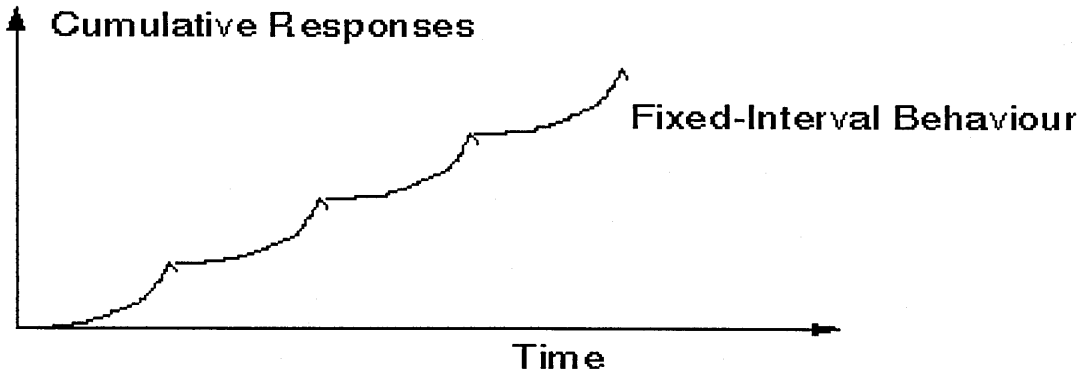

Figure 1.12: After each reinforcer animals respond on FI schedules with gradually accelerating response rates which produces a 'scalloped' record. Adapted from Kentridge (2008).

Interestingly, in food-restricted adolescent (post-natal days 30-45) SHR and WKY, Adriani et al. (2003) observed a shift in preference to the immediate reinforcer from the larger delayed reinforcer as the delay duration increased in all the animals tested. Adriani et al. (2003) found significant inter-individual variability in the SHR in the test for impulsivity. Two distinct subpopulations, showing differences in impulsive behaviour and specific neurochemical parameters, were indicated within the SHR strain. On the basis of the median value of average hole-preference, it was discovered that the 'impulsive' SHR subgroup displayed a very quick shift towards the immediate reinforcer, yet the 'non-impulsive' SHR subgroup produced little or no shift (Adriani et al., 2003). This does raise the possibility that there are subtypes of SHR.

Li et al. (2007) demonstrated an inhibition deficit in the SHR in a prepulse inhibition task. Prepulse inhibition is an examination of the acoustic startle reflex, by assessing sensorimotor gating, or the degree to which a weak acoustic stimulus distracts cognitive processing away from a more prominent acoustic stimulus. It is linked to dopamine levels, and has been heavily utilised in research into schizophrenia. No differences were found between SHR and age- and gender-matched WKY at the lower prepulse stimulus level (Li et al., 2007). At the higher level, SHRs showed profound prepulse inhibition deficits in comparison to the WKY. These results were also reported without any signs of memory

deficits, in line with the findings reporting no differences between the SHR and WKY using a Morris water maze task to assess memory (Li et al., 2007). It further suggests that SHR were more impulsive than WKY, and that differences were not due to a general sensory deficit, as no differences were found at the lower prepulse stimulus level.

1.3.2.2. CONSTRUCT VALIDITY

For an animal model to have construct validity it must be analogous to the human disorder, by having shared neurological substrates and a similar etiology. As the etiology of ADHD is unknown, to establish construct validity in the SHR is speculative, yet similar to individuals with ADHD, monoamine transmission is thought to be affected.

Neurotransmitter dysfunction

Following the evidence for dysfunction in monoamine transmission (see Section 1.1.5) in ADHD , evidence suggests a cortical dopamine hypofunction in the SHR (Russell, 2003). SHR have a decreased turnover of dopamine in the VTA, striatum, and frontal cortex (Linthorst et al., 1994; de Villiers et al., 1995). Young male SHR have an increased density of D1 and D5 receptors in the neostriatum and nucleus accumbens (Carey et al. 1998), and a recent study by Li et al. (2007) showed that SHR show a reduced expression of the D4 receptor gene in the PFC. Interestingly, Warton et al. (2009) found an increased release of dopamine in the SHR in the substantia nigra in comparison to WKY. This is in line with Castellanos (1997) who proposed that both a hyper- and hypo- function of dopamine can be associated with ADHD symptoms, and conclusively, ADHD-like behaviours in the SHR.

It has been proposed that abnormal dopamine function could be an indirect consequence of a dysfunctional glutamate regulation of dopamine neurons (Warton et al., 2009). Warton et al. (2009) found a significant difference in glutamate-stimulated release of dopamine in the substantia nigra of the SHR compared to the WKY, with SHR releasing additional dopamine.

Evidence (Russell, 2003) suggests a selective deficit in the nucleus accumbens shell of the SHR. Thus, dopamine hypofunction could be a secondary effect caused by a defect in the glutamate-stimulated discharge of dopamine in the nucleus accumbens shell of the SHR, and it may play a role in the impaired reinforcement of suitable behaviour observed in the SHR and ADHD sufferers.

In addition to changes in the dopamine system, research has also shown changes in noradrenalin. For example, Russell (2002) demonstrated autoreceptor-mediated inhibition of noradrenalin release was impaired in the prefrontal cortex of the SHR *in vitro*, suggesting that noradrenergic function may be poorly regulated in these animals. Alterations in the noradrenergic system such as elevated concentrations of NA in the LC, substantia nigra and PFC have been found (de Villiers et al., 1995). This finding is in line with an increased NA transmission and a down-regulation of beta-adrenoreceptors (Myers et al., 1981). Glutamatergic-induced NA release in the prefrontal cortex is higher in SHR than in WKY (Russell and Wiggins, 2000), while the stimulus induced release from prefrontal cortex slices does not differ between these rat strains (Russell et al., 2000a, 2000b). However, the inhibition of NA release by the α_2-autoreceptor may be deficient (Reja et al., 2002; Russell et al., 2000a, 2000b; Tsuda et al., 1990) suggesting an overall increased noradrenergic transmission in SHR. Noradrenergic transmission has a crucial function in attentional performance and alert processing of sensory stimuli (Robbins, 2002). Existing neurochemical and pharmacological evidence implies a mild activation of the noradrenergic system causes small enhancements of accuracy in the five-choice serial reaction time task, a test of visual attention (Jakala et al., 1992; Sirvio et al., 1994; Ruotsalainen et al., 1997). Decreased noradrenergic transmission also causes sedation and attentional deficits (Sirvio et al., 1994; Ruotsalainen et al., 1997). Therefore, dysregulation of noradrenalin in SHR and individuals with ADHD may in part cause the attentional deficits observed.

It has also been suggested that serotonin transmission, especially 5-HT_{7R} receptors may play a part in ADHD-like behaviours seen in SHRs. In Sprague-Dawley rats, a mixed serotonin (5-HT_{1a}/ 5-HT_7) agonist cancelled out the increased levels of basal impulsivity produced following administration of a selective 5-HT_{7R} antagonist (Leo et al., 2009). This is also the case with the SHR; basal monoamine levels in various neuronal areas of the SHR were significantly dissimilar from that of WKY (Nakamura et al., 2001). Serotonin turnover was reduced in the SHR, implying a hypofunctional serotonergic system in this animal model. By comparing the typical development of striatal SERT densities of SHR during weaning age until adulthood, Roessner et al. (2009) found an augmented striatal SERT density in older SHR in comparison to WKY, possibly signifying ADHD specific changes in the serotonin system in these animals. Therefore, even though establishing construct validity of ADHD in an SHR is speculative, the animal model does appear to be analogous to the human disorder in regards to dysfunctional monoamine transmission.

Relationship to unifying theories

There are two main unifying theories of ADHD, one that centres around behavioural inhibition (Barkley, 1997) and the other centralising around delay-of-reinforcement gradient and extinction (Sagvolden et al., 2005), as discussed in Section1.1.6. In the SHR there is evidence of behavioural inhibition (see Section 1.1.6), although as the oculomotor paradigms commonly used for this assessment in children (see Section 1.1.6) are not possible in rats, it is difficult to directly compare ADHD-like behaviours seen in the SHR for behavioural inhibition. Altered reinforcement of novel behaviour, as measured by a delay-of-reinforcement gradient and defective extinction are two processes proposed by Sagvolden et al. (2005) to be core behaviours or ADHD. Evidence of both these behavioural processes is evident in the SHR (see Section 1.3.2.1). Evidence of abnormalities of the superior colliculus has also been seen in the SHRs (Dommett and Rostron, 2011; Hernandez

et al., 2003) which could underlie the dopamine dysfunctions explained in the two theories (see Section 1.3.2.2).

Hypertension

The SHR therefore shows several aspects of face and construct validity, however, hypertension is a confounding factor in this animal model because it is not associated with ADHD and it cannot be excluded that increased blood pressure affects behaviour. It has already been stated that the hyperactivity exhibited by the SHR is found prior to the onset of hypertension (Sagvolden et al., 2005). However, the attentional deficits and impulsivity seen in the SHR might reflect dysfunctional processing or brain damage caused by high blood pressure. Indeed, some human studies have shown a negative effect of hypertension on cognition (Anstey and Christensen, 2000; Birkenhager et al., 2001). However, both the sustained attention deficit (Sagvolden et al., 2005) and deficit in impulsivity is seen prior to the development of hypertension in these animals (Sagvolden et al., 2005b). Given the ADHD-like behaviours occur prior to the onset of hypertension, it is likely that they have not arisen as a result of it. In addition, young SHR do not show hypertension and Diana (2002) reported no cognitive decline in aged SHR compared to WKY. These findings suggest that the cognitive deficits in the SHR do not depend on hypertension.

1.3.2.3. PREDICTIVE VALIDITY

Predictive validity refers to the degree to which any measure can predict future or independent past events, such as consistent results that a drug of known efficacy in humans similarly works on the animal model. To develop novel drugs with higher efficacy and fewer side effects, it is essential to have an appropriate predictively valid animal model for *in vivo* drug screening processes.

Psychostimulant pharmacotherapy

The SHR do show some predictive validity to psychostimulants on all core ADHD-like behaviours seen in these animals. Sub-chronic medication with methylphenidate produces behavioural alterations in attention and emotion in the SHR (Pettersson et al., 2011). Similar to the findings of psychostimulant effects on normal subjects, methylphenidate and amphetamine have also been shown to improve sustained attention, and suppresses distractibility behaviours in rats (Bizarro et al., 2004; Evenden and Robbins, 1985; Agmo et al., 1997; Grilly, 2000; Bizarro et al., 2004). Kantak et al. (2008) investigated the effects of oral methylphenidate (1.5 mg·kg-1) on three tasks chosen to measure prefrontal cortical or dorsal striatum function: odour-delayed win–shift (non-spatial working and reference memory), win–stay (habit learning) and attentional set-shifting (attention and behavioural flexibility) tasks. On all three tasks, the SHR made significantly more errors than the WKY. Treating the SHR with methylphenidate eliminated strain differences in all three tasks. Evidence has also shown that hyperactivity is ameliorated by treatment of methylphenidate in SHRs (Sagvolden et al., 1992).

It has been reported that the hyperactivity was ameliorated by treatment of D-amphetamine in SHR (Myers et al., 1982; Sagvolden et al., 1992), suggesting the SHR are predicatively valid for this drug. Sagvolden and Xu (2008) tested ADHD-like behaviour and found D-amphetamine normalised SHR hyperactivity, impulsiveness and sustained attention, but the behavioural effects of L-amphetamine were comparatively more efficient for normalising sustained attention. These findings indicate that hyperactivity and impulsiveness may, in some part, be related to a different imbalance in neural circuits from those that cause poor sustained attention. It suggests that the two amphetamine isomers may affect the neuromodulators in different ways, or suggests that D- and L-amphetamine have different relative potencies on a similar neuronal system.

In contrast to the findings described above, Warton et al. (2009) evaluated behavioural discrepancies in the open field following acute methylphenidate treatment in juvenile SHR and WKY. The SHR were hyperactive in the open field, yet, interestingly methylphenidate administration had no effect on either strain's activity. Interesting, Van den Bergh et al. (2006) found similar results the activity of the SHR was not normalised by methylphenidate, only the WKY showed decreased activity with treatment. SHR also showed no difference in impulsivity following methylphenidate administration, even though the impulsivity of the WIS was improved by the drug (Van den Bergh et al., 2006).

Despite contradictory findings, the SHR do show some predictive validity to psychostimulants, especially for treatment of the attentional deficit which is a core behaviour in this animal model. It is also worth noting that treatment with psychostimulants in individuals with ADHD also has a high non-responder rate of approximately 50% (Newcorn et al., 2008).

Non- psychostimulant pharmacotherapy

Atomoxetine is currently employed to treat ADHD (Spencer et al., 1998). However, few experiments with atomoxetine have been performed in the SHR examining its effects on ADHD behaviours. Turner et al. (2008) found atomoxetine had no effect on locomotor activity or impulsivity when administered orally at 0.1 and 0.3 mg/Kg. Similarly, Dommett (2014) found no significant impact of a range of atomoxetine doses (0.1-5.0 mg/Kg), once multiple comparisons were accounted for on the 5-CSRTT task. However, it is noteworthy that in the latter study, the final sample size was low and therefore insufficient power may have contributed to the lack of effect seen.

1.4.SUMMARY AND RATIONALE FOR THESIS

ADHD is a neurobehavioral disorder of childhood onset, with a prevalence of approximately 3–9% in school-aged children and young people in the UK (National Institute for Health and Care Excellence, 2008). A high percentage of these children have symptoms occurring through adolescence and into adulthood (Faraone et al., 2006, Spencer et al., 2002). Despite clear genetic and environmental risk factors, the etiology of ADHD is unknown. There is, however, evidence that various brain structures are affected and there are dysfunctions in monoamine transmission. In line with this, most ADHD pharmacotherapies act on the dopamine system, although there are concerns that chronic use in the developing brain may cause potential risks of drug abuse and self-control abilities (Macro et al., 2011). The behavioural inhibition theory (Barkley, 1997) and the dynamic development theory (Sagvolden et al., 2005) both claim a role for dopamine is crucial in ADHD development. It could be theorised that the dysregulation of dopamine is a secondary effect of a dysfunction in the initial processing of sensory stimuli, where the saliency of stimuli has been altered. The superior colliculus has direct connections to dopamine neurons (Comoli et al., 2003; McHaffie et al., 2006) and been shown to be capable of activating and modulating midbrain dopamine neuron phasic activity (Dommett et al., 2005; Coizet et al., 2006).

The SC is a sensory-motor structure located on the dorsal surface of the midbrain (Butler and Hodos, 2005; Huerta and Harting, 1984). It has extensive connections with various brain regions, especially with the frontoparietal network. The SC has a wide variety of functions. It is a multimodal structure that integrates information about visual saliency and attentional focus. It also plays a crucial role in saccade generation. So, by integrating information from the frontoparietal network with the assessment of the salience of stimuli, a retinotopic depiction of the relative importance of locations as the subsequent locus for the orientation of attention and gaze is produced by the circuitry in the SC (Fecteau and Munoz, 2006; Dorris et al., 2007; Shen and Pare, 2007; Mysore et al., 2011). Importantly, all

vertebrate species must have the capacity to select information for processing based on its relevance to behaviour. The preservation of the SC throughout evolution suggests it embodies fundamental circuits and mechanisms for competitive stimulus selection and attentional focus in all vertebrates, including humans. The evidence of its connectivity and influence on attentional and motor networks, as well as its central function in behaviours such as saccade generation, visual saliency and attention do suggest that the SC could be dysfunctional in ADHD. Therapeutic effect of psychostimulants on decreasing distractibility and improving sustained attention may arise via an action at the level of the SC. Critically, if this theory is proven correct, it may signify a crucial improvement in the understanding of ADHD and its etiology. There is a theoretical basis for the SC being implicated in the neurobiology of ADHD but research to date has focussed on healthy individuals. As the SC is conserved across species, animal models of ADHD such as the SHR can be used to assess this theory to an extent.

SHR are the main animal model used for ADHD. The animal model does show face validity. Using various behavioural tests, the SHR has been shown to have ADHD-like behaviours such as hyperactivity (Qian et al., 2010), impulsivity (Pardey et al., 2009), deficits in sustained attention and distractibility (Jentsch, 2005). Construct validity is speculative within SHR as the etiology of ADHD is unknown, however, monoamine transmission also seems to be affected in the SHR as in ADHD (Russell, 2003) and the characteristics of the model fit with the two main unifying theories. Predictive validity of ADHD pharmacotherapies in SHR has also been shown (Bizarro et al., 2004), although results are inconsistent (Van den Bergh et al., 2006).

In light of this discussion, the aim of this thesis is to investigate the SC in the SHR model of ADHD. The chapters that follow will address the following hypotheses:

1. There will be behavioural differences in how the SHR responds and habituates to a visual stimulus in an SC-dependent task in comparison to the two control strains (WKY and WIS). (Chapter 3)
2. There will be physiological differences in the responses to visual stimuli recorded in the superficial layers of the SC in the SHR in comparison to the two control strains (WKY and WIS). (Chapter 3)
3. There will be morphological differences in the superficial layers of the SC in the SHR in comparison to the two control strains (WKY and WIS). (Chapter 3)
4. There will be behavioural differences in how the SHR responds and habituates to an auditory stimulus in an SC-dependent task compared to the two control strains (WKY and WIS) . (Chapter 4)
5. There will be physiological differences in the responses to auditory stimuli recorded in the deeper layers of the SC in the SHR in comparison to the two control strains (WKY and WIS). (Chapter 4)
6. There will be morphological differences in the deeper layers of the SC in the SHR in comparison to the two control strains (WKY and WIS). (Chapter 4)
7. There will be a significant effect of amphetamine on visual and auditory responses in the SC in a manner that normalises responses in the SHR, with reference to the WKY and WIS, and additionally the Hooded Lister (HL). (Chapter 5)
8. There will be a significant effect of fluoxetine on visual and auditory responses in the SC in a manner that normalises responses in the SHR, with reference to the WKY and WIS. (Chapter 6)
9. There will be a difference in receptor density of 5-HT_{1A} and 5-HT_{1B} receptors in the SHR strain compared to the two control strains (WKY and WIS). (Chapter 6)

2. MATERIALS AND METHODS

This chapter details the key methods used to characterise the behavioural, physiological and morphological aspects of the superior colliculus in the SHR and control strains. Further specific procedural and analytical details, including subject numbers for specific experiments, as well as any variations on these methods, are given in the individual experimental chapters.

2.1.SUBJECTS

Adult male rats (Harlan Laboratories Inc., UK and Charles River Laboratories, Germany) aged 15-20 weeks at the start of testing were housed within the Biomedical Resource Unit (BRU) at the Open University. Rats were housed in groups of three within scantainers held at a constant temperature of 21-23 °C. The holding room was on a 12:12hr reverse light/dark cycle with lights going off at 8am and turning on at 8pm. Rats were given at least one week to habituate to the BRU after arrival from the supplier prior to use in any experiments. All experiments were carried out in the dark phase and therefore at the time when rats are most active. Standard laboratory rat chow (RM3 diet from Special Diet Services, Witham, UK) and water were available *ad libitum* throughout all experiments. All procedures were conducted in accordance with the Animal (Scientific Procedure) Act (1986) and local ethical review requirements. A timeline indicating what happened to a typical experimental animal is illustrated in Figure 2.1. Potentially an animal could follow one of three timelines. Animals were either used for electrophysiological experiments only before post-mortem processing, yet some animals were used for the behavioural tasks prior to electrophysiological experiments. A small selection of animals were used for the morphological experiments only and were not included into any other protocol.

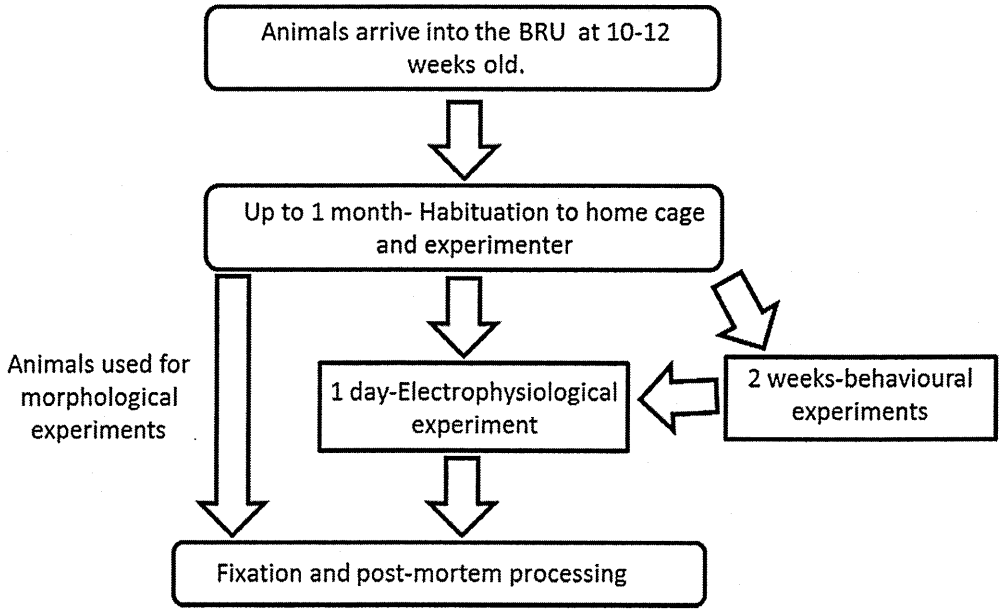

Figure 2.1: A timeline indicating what happened to the experimental animals used in this thesis.

The total number of rats used for all experiments was 153. Table 2.1 shows the breakdown of this total into the four different strains used (Spontaneously Hypertensive Rat, SHR; Wistar Kyoto Rat, WKY; Wistar Rat, WIS; and Hooded Listar Rat, HL) and the average weight of the rats for each strain recorded immediately prior to any experimental procedure. Note that the HL was only used for experiments investigating the effects of amphetamine (see Chapter 5) to allow better comparison to existing literature and therefore fewer of this strain were required. The normality of the weight data was confirmed using the Kolmogorov–Smirnov test and a One-Way ANOVA was then used to reveal a significant difference in weight between the strains ($F=37.03$; $df=3$; $p=0.0005$). Post-hoc (Tukey HSD) tests indicated that the WIS had a significantly greater weight than the WKY ($p=0.0005$), SHR ($p=0.0005$) and HL ($p=0.002$). The HL also had a significantly greater weight than the WKY ($p=0.043$), and the SHR ($p=0.033$). There was no significant difference between the WKY and SHR ($p=0.999$).

Strain	Total Number	Mean weight ± SEM (g)
SHR	47	395.04±4.79
WIS	46	494.40±10.0
WKY	45	396.31±7.81
HL	15	437.47±10.50

Table 2.1: The total number and mean weight ± SEM of the adult male rats used. WIS had a significantly greater weight than the other 3 strains, and the HL had a greater weight than the SHR and WKY.

2.2. BEHAVIOURAL TESTING

All stages of the behavioural experiments were carried out between the hours of 9am and 5pm. All animals were habituated to the experimenter prior to behavioural testing to reduce any stress response which would otherwise impact on locomotor activity (Williams and Russell, 1972) and therefore on the behavioural measures. This habituation took the form of daily handling of all animals by the experimenter for approximately five minutes for a period of one month (not including weekends) prior to testing. This long period of habituation was deemed necessary because of the increased anxiety of the WKY (McAuley et al., 2009). There was additional habituation to the testing equipment as detailed below for the individual tests.

All testing was carried out in a dimly red-lit room, using automated data collection or video equipment and a remotely controlled experimental paradigm so that the presence of the experimenter did not disrupt the animal's behaviour. Extraneous sensory cues, which could serve to alter the animals' behaviour, were removed where possible by using a white noise generator to block out any background sounds and disinfectant to remove olfactory cues from the testing apparatus between animals (Langley, 1993).

2.2.1. DISTRACTION TASK

A task measuring initial responses and habituation of responses to a distracting stimulus was used as a measure of behaviour dependent on the superior colliculus (Clements et al., 2010). The task examines responsiveness to a series of unexpected sound tones or light flashes,

an important function of the SC, to determine whether a change in responsiveness or the ability to habituate to the stimuli may underlie distractibility in the SHR.

Procedure

For this task animals were placed in a circular arena, approximately 2.5 m diameter with the stimulus centrally located as shown in Figure 2.2. All rats were tested with both auditory and visual stimuli.

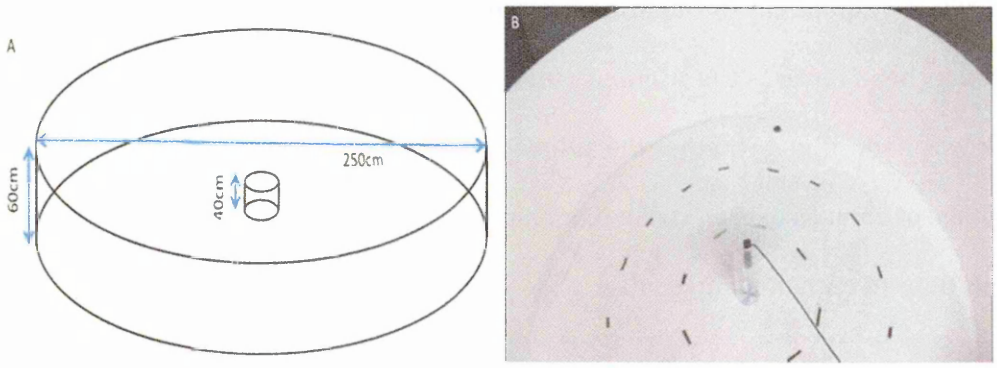

Figure 2.2: The stimulus was positioned in the middle of the arena within in a Perspex box and controlled remotely. A: a diagram and B: a photograph of the circular arena used to assess distractibility.

The order of testing was counterbalanced to strain, as well as stimulus modality to remove any order effects. The experiment was conducted over three consecutive days for each modality. On each of the first two days the animal was placed in the arena for 15 minutes to allow habituation to the environment. On the third day, the animal was placed in the arena and, following a 5 minute interval, a light flash (20 Mcd) or a tone (70 dB SPL) was produced for 5 seconds. This occurred ten times at 5 minute intervals with the same stimulus modality. Thus, the animal stayed within in the arena for approximately one hour during the trial for one stimulus modality. The animals then received a break of one week before the second three day period began to test the other modality. Therefore, in total the animals were exposed to the arena on six days, two of which were under experimental conditions, once for each modality. The animal's behaviour was recorded using a video camera (Samsung VP-HMX20C) aerially viewing the whole arena. The camera and stimulus

presentation was controlled from outside of the room, meaning that the experimenter was not present during behavioural trials and therefore could not impact on the animal's behaviour. The videos were then stored to a PC for subsequent analysis.

Data analysis

Video data were analysed in two ways for each stimulus modality. Firstly, a decision was made as to whether the animal responded to each stimulus presentation. An animal was perceived as responding to the stimulus if it physically interacted with the stimulus, or oriented its head towards the stimulus (see Figure 2.3) or froze in response to the stimulus. Once it was determined whether the animal had responded it was possible to calculate the percentage of animals of each strain that responded for each of the ten consecutive stimulus presentations in each modality.

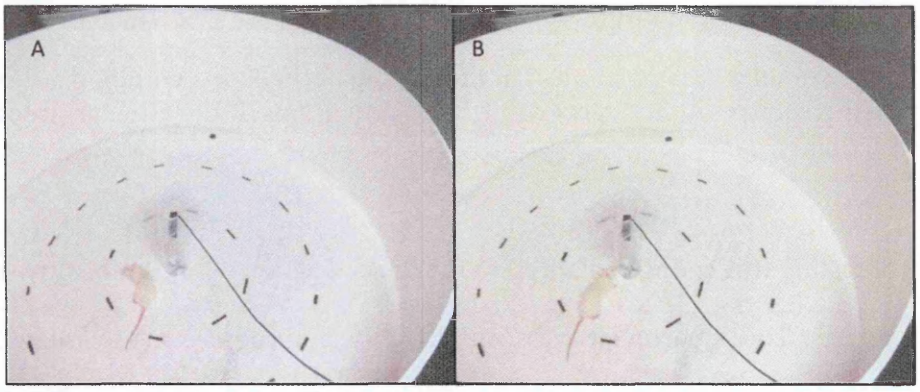

Figure 2.3: Examples of an animal's behaviour within the arena. A: the animal is orientating away from the stimulus (deemed as no response to the stimulus). B: the animal is interacting with the stimulus (deemed as responding to the stimulus).

Secondly, the duration of response to the stimulus, whether physically interacting, head orienting or frozen, was measured for each stimulus during the 5 seconds in which the stimulus was on and expressed as a percentage of that time. As well as the duration of response within the 5 seconds while the stimulus was on, the 5 second pre- and post-stimulus periods were also included in analysis to assess whether the animals were affected by the stimulus when it was not actually on. That is, if their behaviour was a general behaviour directed towards the object rather than sensory stimulus. The assessments of

these periods were repeated for the 10 consecutive stimulus presentations to assess differences in the duration of response, by strain, over repeated presentations.

All data were confirmed as having a normal distribution using the Kolmogorov–Smirnov test before being analysed using a repeated measures ANOVA with STRAIN as the between-subjects factor, and STIMULUS PRESENTATION as the within-subjects factor. Where the sphericity assumption was violated the Greenhouse Geisser correction was used.

2.2.2. LOCOMOTOR ACTIVTY

Despite only adult animals being used throughout the experiments, it should be mentioned that juvenile SHRs are hyperactive in familiar but not novel environments (Li et al., 2007; Pardey et al., 2009). Increased or altered locomotor activity could confound any measures of distractibility and therefore locomotor activity was monitored in the same group of animals used for the distractibility test during the same two week period to ensure locomotor activity was not different between the strains and therefore ensuring it was not a confounding variable. The results of this test are provided below and referred back to in the experimental chapters because they are a check for a confounding variable rather than a primary result.

Procedure

Locomotor activity was monitored using automated Activity Monitoring Chambers (Med-Associates, UK) consisting of Perspex chambers, measuring 45 cm (width) by 45 cm (length) by 30 cm (height), with infrared beams automatically detecting horizontal and vertical movement (see Figure 2.4).

Figure 2.4: Photographs of the automated activity monitoring chambers; movement was automatically detected using horizontal and vertical infrared beams.

As with the distractibility task, testing was conducted over three consecutive days. On the first two days, animals were habituated to the locomotor chambers for 15 minutes each day, thus ensuring a familiar environment in order to assess hyperactivity. On the final day, locomotor activity was monitored for 30 minutes while a series of measurements were automatically recorded for analysis off-line. The measurements automatically taken in five minute epochs for statistical analysis were (i) "distance travelled" - the total horizontal distance moved in cm; (ii) "average velocity" - average horizontal velocity in cm/min; (iii) "vertical activity"- the number of continuous vertical beam breaks indicating rearing; (iv) "stereotypic activity"- the number of partial-body movements that happened within a defined space, such as grooming, head-weaving or scratching movements; and finally, (v) "jumps"- the number of jumps measured by when the rat left the photo beam array for a period of time.

All data from the locomotor activity test were confirmed as having a normal distribution using the Kolmogorov–Smirnov test before analysis with repeated measures ANOVA with STRAIN as the between-subjects factor and TIME as the within-subjects factor. Where the sphericity assumption was violated the Greenhouse Geisser correction was used.

There was no main effect of STRAIN for average velocity ($F=0.66$; $df=2, 0.05$; $p=0.528$), stereotypic activity ($F-0.44$; $df=2, 0.04$; $p=0.650$) and jumps ($F=2.25$; $df=2, 0.16$; $p=0.128$). However, there was a main effect of STRAIN on distance travelled ($F=4.10$; $df=2, 0.26$; $p=0.030$). Post hoc (Tukey HSD) analysis revealed that there was only a trend towards the WKY moving a significantly less distance than the WIS ($p=0.052$) and SHR ($p=0.056$), and no significant difference between the WIS and SHR ($p=0.994$). There was also a main effect of STRAIN on vertical activity ($F=4.12$; $df=2, 0.26$; $p=0.029$). Post hoc (Tukey HSD) analysis showed the SHR were significantly more vertically active than WKY ($p=0.023$) but not the WIS ($p=0.480$). There were no significant differences between WIS and WKY ($p=0.265$).

There was a main effect of TIME for four of the five parameters (distance travelled: $F=67.46$; $df=5, 0.75$; $p=0.0005$; stereotypic activity: $F=31.57$; $df=4.05, 0.58$; $p=0.0005$; jumps: $F=8.84$; $df=3.10, 0.28$; $p=0.0005$; and vertical activity: $F=11.02$; $df=3.07, 0.32$; $p=0.0005$) and a trend towards a main effect of TIME on average velocity ($F=2.38$; $df=3.63, 0.09$; $p=0.064$). Within-subjects contrasts revealed there was a significant decrease in all parameters at the first to second epoch (distance travelled: $F=118.53$; $df=1, 0.84$; $p=0.0005$; stereotypic activity: $F=36.71$; $df=1, 0.62$; $p=0.0005$; average velocity: $F=10.34$; $df=1, 0.031$; $p=0.004$; jumps: $F=10.06$; $df=1, 0.30$; $p=0.004$; vertical activity: $F=22.04$; $df=1, 0.49$; $p=0.0005$). This was the only significant difference between each epoch for average velocity, both showing no significant differences at any other epoch. There was a significant decline in stereotypic activity up to and including the third compared to the fourth epoch

(F=4.72; df=1; p=0.040), yet at the final two epoch there was no further change. There was a significant difference in jumps up to and including the fourth to fifth epoch (F=7.80; df=1; p=0.010), yet at the final epoch where there was no further change. With increasing time in the chamber, there was a consistent decline in distance travelled, up to and including a significant decline in distance travelled between the fifth to final epoch (F=34.41; df=1; p=0.0005). These results are expected as they correspond to a general decrease in exploratory behaviour as they became increasingly familiar with the environment.

There was no TIME x STRAIN interaction for average velocity (F=1.02; df=7.25, 0.08; p=0.423), stereotypic activity (F=1.63; df=8.11, 0.12; p=0.125) and vertical activity (F=2.08; df=6.15, 0.15; p=0.070). There was a TIME x STRAIN interaction for the distance travelled (F=5.15; df=10, 0.31; p=0.0005) and jumps parameter (F-2.34; df=6.19, 0.17; p=0.039) (Figure 2.5).

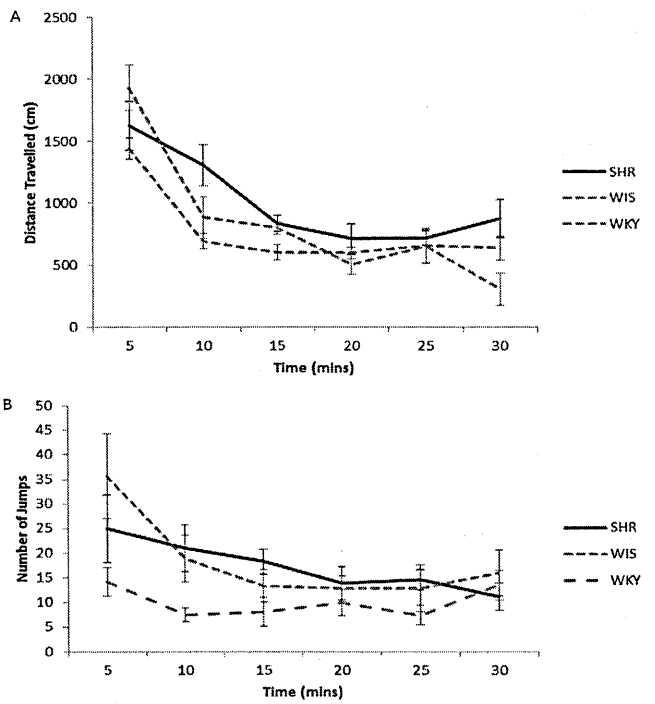

Figure 2.5: The mean ± SEM A: distance travelled and B: jumps of the three strains with increasing time showing a significant main effect of time, with decline in distance travelled and jumps as the time increased. There was a main effect of strain for distance travelled only where the WKY showed a trend towards travelling less distance than both the WIS and SHR. There was an interaction between strain and time for both measured (see text for detailed description).

Restricted repeated measures ANOVAs were used to investigate the interaction effect for the distance travelled, restricted by STRAIN first of all. Examination of Figure 2.5a indicates there is no interaction between the SHR and WKY because these lines remain largely parallel. A restricted analysis including only these two strains showed this to be the case because there was no significant interaction ($F=1.76$; $df=5$; $p=0.131$). Further restrictions excluding the WKY and SHR in turn showed there were significant interactions between the WIS and SHR ($F=5.59$; $df=5$; $p=0.0005$) and the WIS and WKY ($F=7.23$; $df=5$; $p=0.0005$) indicating that it is the differences in distance travelled between the WIS and the other two strains responsible for the overall interaction effect. In order to find exactly where these interactions occurred, further restricted repeated measures ANOVAs, this time restricted by TIME were conducted for the two strain comparisons (SHR vs WIS and WKY vs WIS). Figure 2.5a indicates that the SHR and WIS interaction is likely to be between the first and second time point where the WIS have a greater decline than the SHR. A restricted analysis removing the first time point removed any significant interaction ($F=1.01$; $df=4$; $p=0.412$). Further restricted analyses removing any of the other time points did not impact on the interaction effect indicating the significant interaction between SHR and WIS is due to their differences in distance travelled in the first and second epoch (i.e. the first 10 minutes). For the interaction between the WIS and the WKY, Figure 2.5a would suggest that the interaction lie either between the first and second time points, with the WIS showing a greater decrease, or between the final two time points where the two strains show opposing changes. Restricting the ANOVA to remove the first time point did impact on the significance of the interaction effect ($F=2.65$; $df=4$; $p=0.042$) reducing it considerably but not fully removing it. However, no other restrictions impacted on the interaction effect suggesting that the greater decrease in the WIS distance travelled between the first and second time point was the cause of the main interaction between these strains.

Restricted repeated measures ANOVAs were used to investigate the interaction effect for the jumps parameter, restricted by STRAIN first of all. Examination of Figure 2.5b does not give a clear indication of where the interaction may lie and therefore restriction by strain was conducted for all combinations. There was no significant interaction between the SHR and WIS ($F=1.49$; $df=2.82$; $p=0.231$). There was a trend towards a significant interaction between the SHR and WKY ($F=2.73$; $df=2.99$; $p=0.054$). Finally, there was a significant interaction between the WIS and WKY ($F=3.14$; $df=2.84$; $p=0.038$) indicating that it is the differences in jumps between the WKY and the other two strains responsible for the overall interaction effect. In order to find exactly where these interactions occurred, further restricted repeated measures ANOVAs, this time restricted by TIME, were conducted for the two strain comparisons (SHR vs WKY and WKY vs WIS). Figure 2.5b indicates that the WKY and WIS interaction is likely to be between the first and second time point where the WIS have a greater decline than the WKY. A restricted analysis removing the first time point removed any significant interaction ($F=1.12$; $df=3.04$; $p=0.351$) between these two strains. Further restricted analyses removing any of the other time points did not impact on the interaction effect indicating the significant interaction between WKY and WIS is due to their differences in jumps in the first and second epoch (i.e. the first 10 minutes). For the interaction between the SHR and the WKY, Figure 2.5b would suggest that the interaction lie between the final two time points where the two strains show opposing changes. Restricting the ANOVA to remove the final time point removed any significant interaction ($F=1.20$; $df=2.72$; $p=0.318$). No other restrictions impacted on the interaction effect suggesting that the opposing change in jumps in the two strains between the final two time points was the cause of the main interaction between these strains.

2.3. *IN-VIVO* ELECTROPHYSIOLOGY

2.3.1. GENERAL SURGICAL PREPARATION

Animals were anaesthetised by an intraperitoneal injection of 30% urethane (1.5 g/Kg, Sigma, UK) solution administered at a volume of 5 ml/Kg. Anaesthetic depth for surgery was assessed by loss of the pedal reflex and eye blink reflex. Body temperature was measured throughout the experiment using a rectal thermometer connected to a thermostatically-controlled heating blanket (Harvard Apparatus Ltd, UK) to maintain temperature at 36-38 °C.

Once the animal was suitably anaesthetised and positioned on the heating blanket, both eyes were sutured open and liquid tear gel (Viscotears ®, Novartis Pharmaceuticals Ltd., UK) applied to prevent desiccation. The animal's head was shaved, and positioned into the stereotaxic frame (Kopf Instruments, Tujunga, USA) using modified ear-bars containing speakers to secure the head (Sheffield University, UK). The head was fixed with the incisor bar 3.3 mm below the interaural line in the skull flat position. Following local anaesthetic (Ethyl Chloride BP, Cryogesic ®, Acorus Therapeutics Ltd., UK) application to the scalp, scalp retraction, and a craniotomy and durotomy was performed, creating two 3 mm Ø burr holes exposing the cortex above the superior colliculus (right electrode:-6.3 mm AP to Bregma, and +2 mm ML to the midline; left electrode: -6.3 mm AP to Bregma; and +3.5 mm ML to the midline; Figure 2.6) to allow for simultaneous recordings form both superior colliculi. This required the left arm to be positioned at a 25° angle from the vertical (see Figure 2.7), whilst the right arm was positioned vertically. This resulted in the right tungsten electrode (A-M Systems Inc, USA) being positioned directly above the superficial layers of the superior colliculus (-6.3 mm posterior to Bregma, and +2 mm lateral to the midline) and it was initially lowered to approximately 1.5 mm ventral to the cortical surface (Paxinos and Watson, 1998; Figure 2.6). On the left side, the tungsten metal electrode was

directed stereotaxically into the superficial layers, from +3.5 mm lateral to the midline at a 25° angle and was initially lowered to approximately 2 mm ventral to the cortical surface (Paxinos and Watson, 1998).

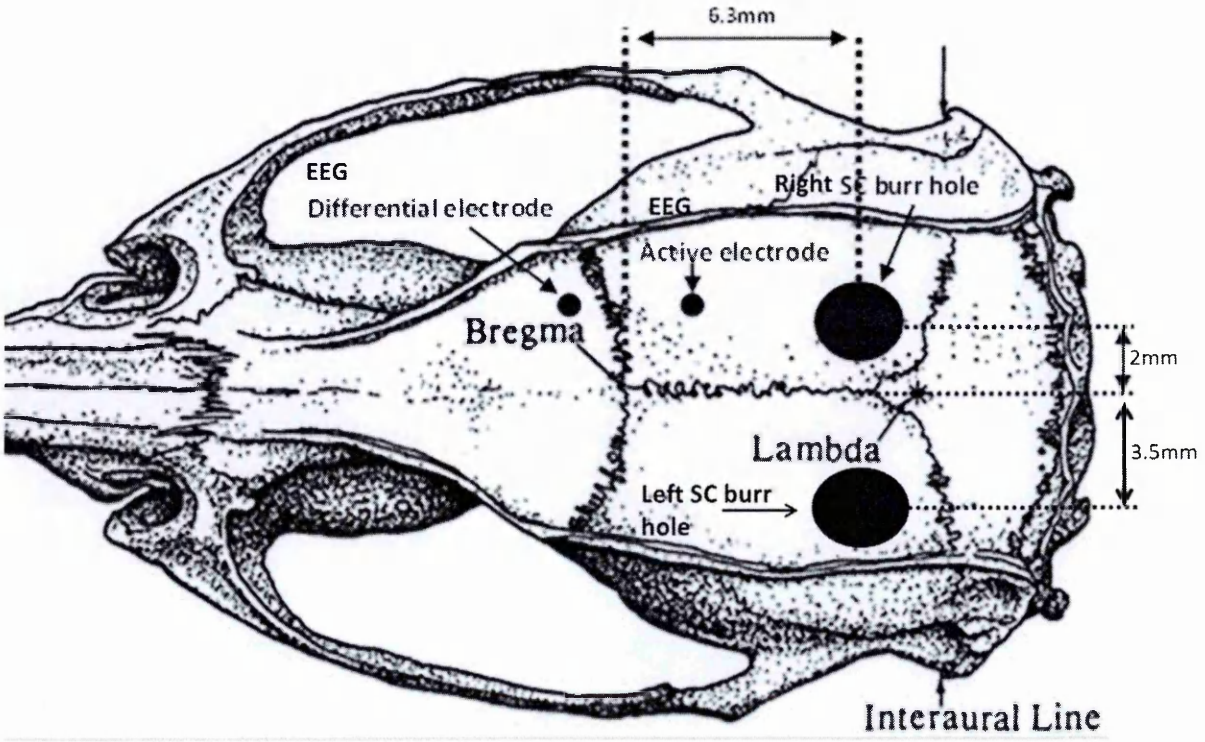

Figure 2.6: Position of Bregma, lambda, and the midline as identified by Paxinos (Adapted from Paxinos and Watson, 1998). Location of SC burr holes, and the two trepanned holes for the EEG.

In addition, two trepanned holes (1 mm Ø) were created anterior to the SC burr holes at specific stereotaxic co-ordinates for electroencephalographic (EEG) recordings (+1 mm anterior, +2 mm lateral; and -4mm posterior, +3mm lateral, relative to Bregma, Figure 2.5.) (Devonshire et al., 2009). Differential and active EEG electrodes (loop-tipped silver wire, 0.2 mm Ø; Intracel) were placed ~1 mm subcranially into the rostral and caudal trepanned holes, respectively to obtain continuous EEG information. Finally, respiration rate was recorded using a three-axis accelerometer IC (ADXL330KCPZ, Analog Devices, Norwood, MA, USA, device (Oxford University, UK)), attached to the animal's lateral abdomen (Devonshire et al., 2009).

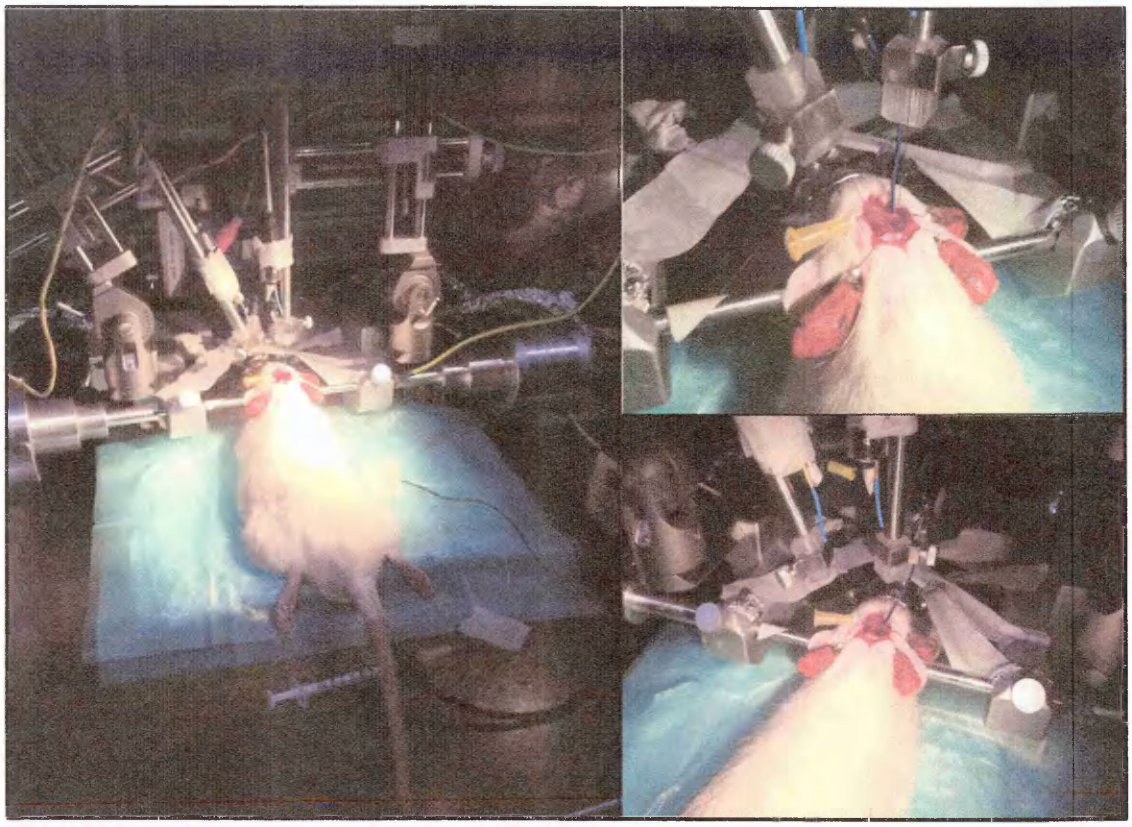

Figure 2.7: Photographs of the electrophysiology experimental set-up. The ear bars are also the speakers, and the LED lights are positioned 5 mm in front of both eyes. The left headstage is angled to allow for two electrodes and thus simultaneous recordings from both sides of the superior colliculus.

2.3.2. FEMORAL VEIN CANNUALTION

Where administration of drug or saline was required, this was done intravenously (i.v.). Prior to the animal's placement in the stereotaxic frame, the right femoral vein was cannulated. Cannulation material consisted of a single length (200 mm) of sterile microenethane tubing (Smith's Medical International Ltd, UK) with a 0.4 mm bevelled intra-vascular tip. A 25 gauge needle (Becton Dickinson Ltd, UK) was inserted into the other side of the tubing; to allow for easy attachment of a syringe with the relevant solution.

The anesthetised animal was placed in dorsal recumbency and the surgical area shaved and the skin swabbed with alcohol. A 2 cm ventral skin incision was made along the crease formed by the abdomen and right thigh (see Figure 2.8). Blunt dissection of the adductor

muscles was used to visualise the right femoral vein. The vein was separated from surrounding tissue as well as the artery and sciatic nerve. Two pieces of equal length silk suture (KeeboMed, Inc, USA) were passed beneath the vein. With reference to the heart, the distal suture was tied tightly to occlude blood flow from the leg and the proximal suture was tied loosely around the vein. A vein clamp (Fine Scientific Instruments, Germany) was also secured around the vein at this proximal location. A small incision was made in the femoral vein between the two ligatures using a 25 gauge sterile needle (Becton Dickinson Ltd, UK). The bevelled end of a catheter filled with sterile saline solution (0.9%) was gently introduced into the vein for a pre-determined distance dependent on the size of the rat; and the loose ligature was tightened around the catheter and vein to secure the catheter in place. Following this, the vein clamp was removed, and patency was confirmed by the appearance of blood easily flowing into the catheter on pulling back of the syringe. After confirming patency, the catheter was secured using sutures, and the skin incision closed. The catheter was filled with sterile heparin (6 mg in 100 ml saline) solution and 0.1 ml was administered through the cannulation to prevent blood clots once the viability of the cannulation was confirmed.

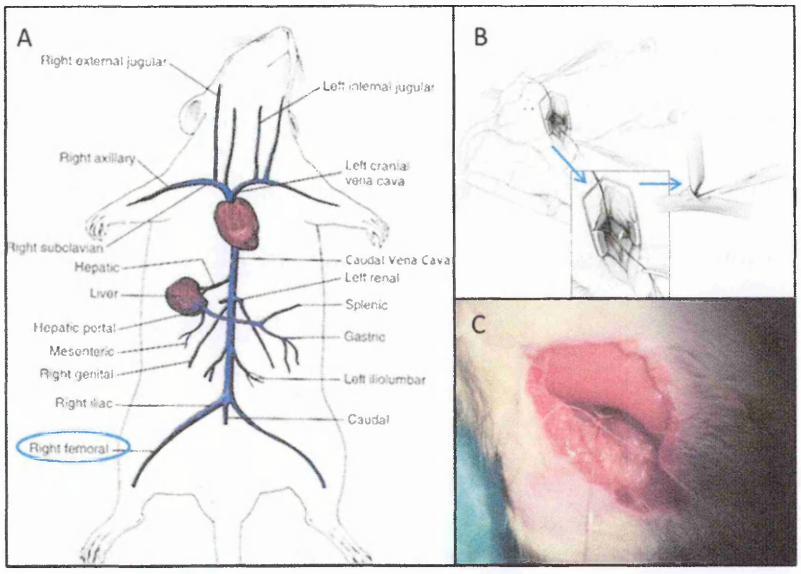

Figure 2.8: A: A diagram of the key veins in the rat's cardiovascular system; B: A step-by-step diagram of procedure used to insert the cannulation into the femoral vein; C: A photograph of the femoral vein cannulated (adapted from biologycorner.com).

2.3.3. DRUG ADMINISTRATION

Following recordings of baseline responses for 300 stimulations, a cumulative dosing regime was used to administer drug doses intravenously via the previously inserted cannula into the femoral vein for two of the experiments described in this thesis. For amphetamine (Sigma Aldrich, UK) and fluoxetine (Tocris Biosciences, UK) cumulative doses (see Table 2.2) or the volume equivalent saline injections were administered with each dose separated by twelve minutes. This allowed for a two minute pre-stimulation period during which the drug took effect and then a ten minute period to collect recordings during 300 stimulations with either a visual or auditory stimulus. With the 1st intravenous injection, an extra 0.06 ml of solution was added to allow for the excess solution in the cannulation tube. More detail of doses, and effects of these drugs are discussed in the relevant chapters (Chapter 5 and 6).

Accumulative Dose	Amphetamine (mg/Kg)	Fluoxetine (mg/Kg)
1st	0.5	0.625
2nd	1	1.25
3rd	2	2.5
4th	4	5
5th	8	10

Table 2.2: The cumulative doses of drug administered.

2.3.4. POSITIONING OF RECORDING ELECTRODES

In order to position the electrodes within the appropriate layers of the superior colliculus, a light flash stimulus was used to identify the superficial layers in turn for each side of the brain. The light (green LED flashing at 0.5 Hz, 10 ms duration, 4-20 Mcd) was positioned directly (5 mm distance) anterior to the contralateral eye, to ensure a whole light field response was produced. The electrode was lowered from its initial position following the surgical preparation on the dorsal surface of the superficial layers until a strong light response was detected. Depending on the stimulus modality being examined, the electrode either remained at this level for recording responses to visual stimuli or was lowered further until the light response was abolished and an auditory response was found within

the deeper layers of the superior colliculus. The speakers delivering the auditory stimulus (8 KHz tone sounding at 0.5 Hz, 50 ms duration, 55-75 dB SPL) were already positioned, as they acted via modified ear bars (see Figure 2.7). For both types of stimulus response the recordings were listened to via a speaker (NL120, The Neurolog System, Digitimer, UK), and watched in real time (see Figure 2.9) to allow for accurate placement. The stimulus modality, strain and side of recordings were counterbalanced throughout.

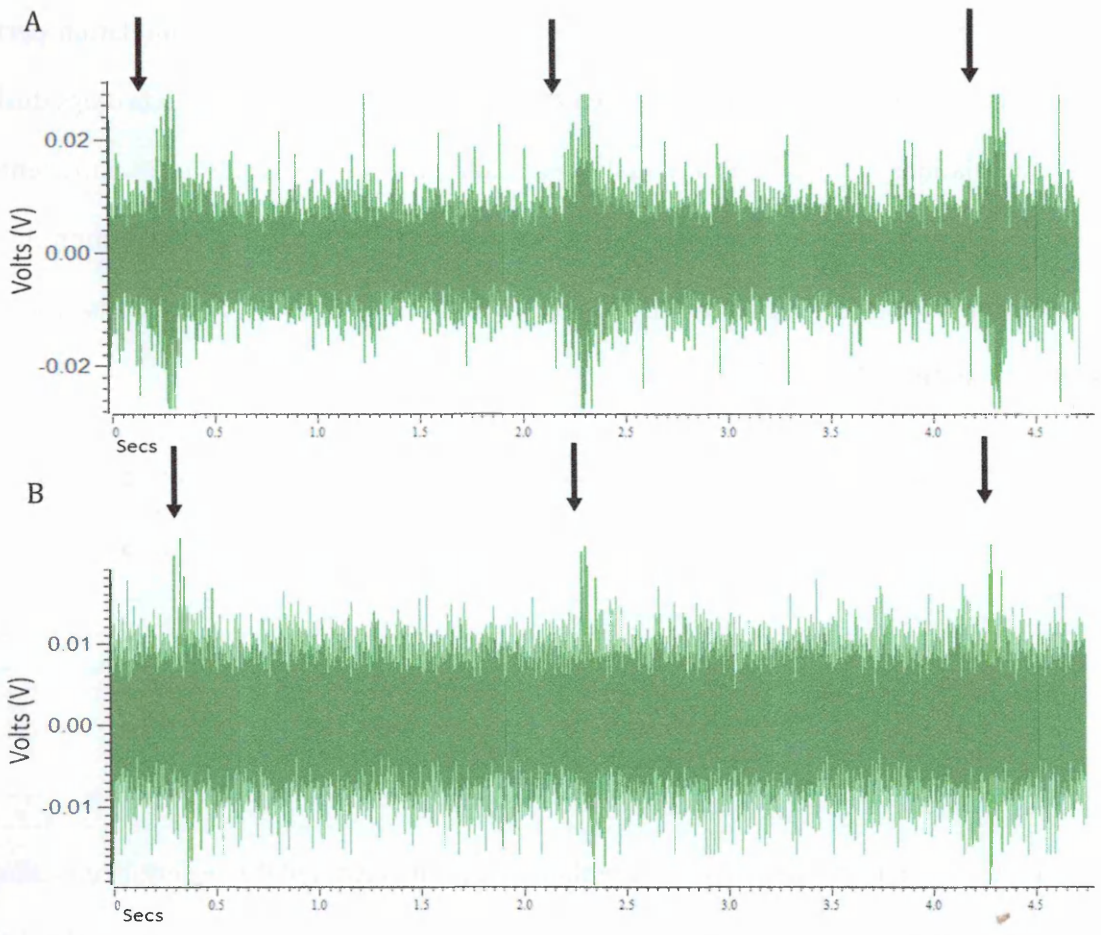

Figure 2.9: An extract from a raw trace showing 3 consecutive typical multiunit responses A: to a whole field light in the superficial layers of the SC. B: to an auditory stimulus in the deeper layers. The stimulus occurred at the time indicated by the black arrows.

When the electrodes were positioned appropriately, the animal was left in the dark for a further 25 minutes to adapt to the darkness before actual recordings began. The visual or auditory responses from 300 stimulations were then recorded at 5 different stimuli intensities (from minimum to maximum light: 4 Mcd, 8 Mcd, 12 Mcd, 16 Mcd and 20 Mcd

and tone: 55 dB SPL, 60 dB SPL, 65 dB SPL, 70 dB SPL and 75 dB SPL) for offline analysis. This allowed a stimulus response curve to be produced and the intensity which gave a mid-range response to be selected for the drug administration experiments. Multiunit recordings from both superior colliculi were amplified at a gain of 1 KHz (NL104, The Neurolog System, Digitimer, UK), band pass filtered (NL125/6 The Neurolog System, Digitimer, UK) between 0.4 KHz-16 KHz, digitised at 11 KHz and recorded directly onto a computer disk using a 1401 hardware acquisition system (CED, Cambridge, England) connected to a PC running with CED Spike2 software (version 7.00).

2.3.5. DATA ANALYSIS

It should be noted that all data analysis was conducted blind to the strain of the animal used to ensure unbiased analysis of data.

Anaesthetic depth

Both EEG and respiration rate were used throughout to assess anaesthetic depth but also used for offline analysis to ensure there were no strain differences in depth that could confound the results. The EEG signal was sampled at 2500 Hz, high-pass filtered from 0.5 Hz (NL125/6 The Neurolog System, Digitimer, UK), amplified at a gain of 1 KHz (NL104, The Neurolog System, Digitimer, UK), and saved directly to the computer for on and offline frequency analysis using Spike2 software as a digitalised signal via a microCED1401 data acquisition unit (Cambridge Electronic Design, Cambridge, UK). The respiration signal from the accelerometer was sampled at 1000 Hz, low-pass filtered at 10 Hz (Oxford University, UK) and also digitized via a microCED1401 for analysis offline.

In order to characterise anaesthetic depth, the dominant EEG frequency was obtained using a power spectrum analysis (Spike2 software) for the period within which the 300

stimulations where presented. The respiration rate per minute was calculated during the first and last 30 seconds of each stimulation period and then used to calculate an average rate per minute over the whole recording period. A breath was counted as every time the respiration trace rose above a pre-set threshold (see Figure 2.10).

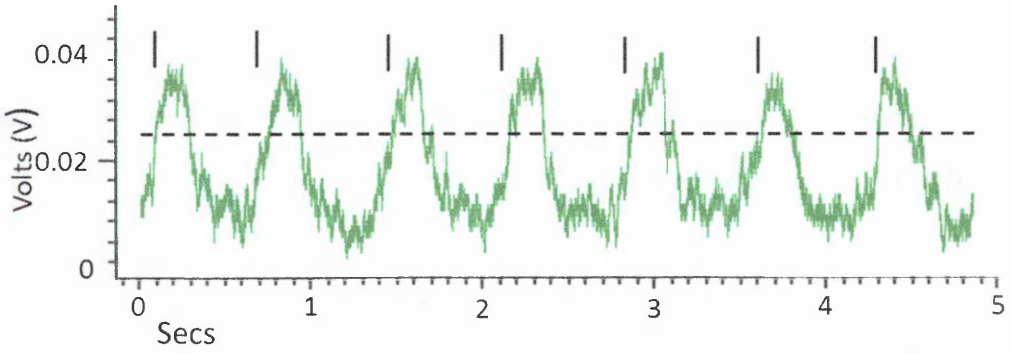

Figure 2.10: A raw trace of the respiration rate and the threshold of the counter indicated by the dotted line. When the trace rose above the line a breath was recorded.

Using Kubicki's (1968) EEG frequency bands and Friedberg et al. (1999) respiration rates, anaesthetic depth could be inferred using Guedel's (1920) stages of anaesthesia for urethane anaesthesia (see Table 2.3).

Guedel Stages of Anaesthesia	Kubicki's EEG bands	Friedbergs's Respiration rats (breaths/min)
III-1	10-13 Hz	NA
III-2	5-7 Hz	96-120
III-3	3-4 Hz	88-104
III-4	1-2 Hz	48-68
IV	Suppression	24-38

Table 2.3: Dominant EEG and respiration rates correlated to Guedel stages of anaesthesia by a rat under urethane. Adapted from Friedberg et al. (1999).

Based on the EEG frequency bands all animals were found to be in stage III-4, with an EEG frequency of 1-2 Hz (see Table 2.3) during recordings. However, the respiration rates did not correspond to this stage of anaesthetic depth according to Friedberg et al. (1999), who suggested that an adult male rat in III-4 anaesthetic depth under urethane should have a respiration rate of between 48-68 breaths/min. Rather, we found considerably higher respiration rates for all strains: Wistar 101±8.19 breaths/min; WKY 88±3.61 breaths/min;

SHR 74±8.54 breaths/min and HL 92±7.54 breaths/min. This increased rate is likely to be in part due to the greater weight of the animals used in this study in comparison to the work by Friedberg et al. (1999) which used Long-Evans (hooded) rats weighing between 250g-300g. In any event, it was most critical to establish that there were no difference in anaesthetic depth between strains and therefore once all data was confirmed to have a normal distribution using the Kolmogorov–Smirnov test, and a One-Way ANOVA ($F=3.52$; $df=3$; $p=0.098$) was conducted to show no significant difference in average respiration rate between the strains.

Collicular recordings

In addition to the amplification and filtering of multiunit recordings in the colliculi outlined in Section 2.3.4, the data from both electrodes were high pass (>1 KHz) and low pass (<1 KHz) IIR digitally filtered (Butterworth model; order 10) offline in Spike2 software to separate the data into local field potentials (low pass <1 KHz) and multiunit activity (high pass >1 KHz) channels, which were dealt with separately. Local field potentials are considered as the synchronised input into the recording space, as high frequencies are filtered out, slower frequencies representing the postsynaptic potential, (i.e. excitatory postsynaptic potentials and inhibitory postsynaptic potentials) are kept for analysis. Similarly, the multiunit activity data represents the output from the area. The fast frequencies are mostly caused by the short inward and outward currents of action potentials, representing the spike activity of neurons.

Local field potentials

Waveform averages were produced for each set of 300 stimulations. Each waveform average was 2.0 s in duration, with a bin width of 0.001 s extending from time T=-100ms to +1900 ms, with the stimulus presentation at time T=0. The period between T=-100 and T=0 was taken as a pre-stimulus baseline. The data from the waveform average was imported into a custom-made Excel macro (Peter Furness, Sheffield University, UK) for analysis of

response parameters. A response was deemed to have occurred if the trace extended beyond a pre-determined threshold after stimulus onset. The threshold for change was set at ±1.96 standard deviations from the mean baseline (i.e. within 95% confidence levels). This threshold was used to assess three parameters: onset latency, peak-to-peak amplitude and duration (see Figure 2.11). Onset latency was obtained by recording the time after stimulus presentation at which the voltage trace extended beyond the threshold. Response duration was determined by obtaining the time, post-stimulus, when the voltage trace returned to within baseline levels (i.e. ±1.96 standard deviations of the pre-stimulation mean) and consistently stayed below this value for 10 ms or 10 bins. The time between onset latency and the response finishing was then used to calculate duration. Finally, peak-to-peak amplitude was defined as the voltage difference between the maximum positive peak and the maximum negative peak in the response period defined by the significant deviation from baseline.

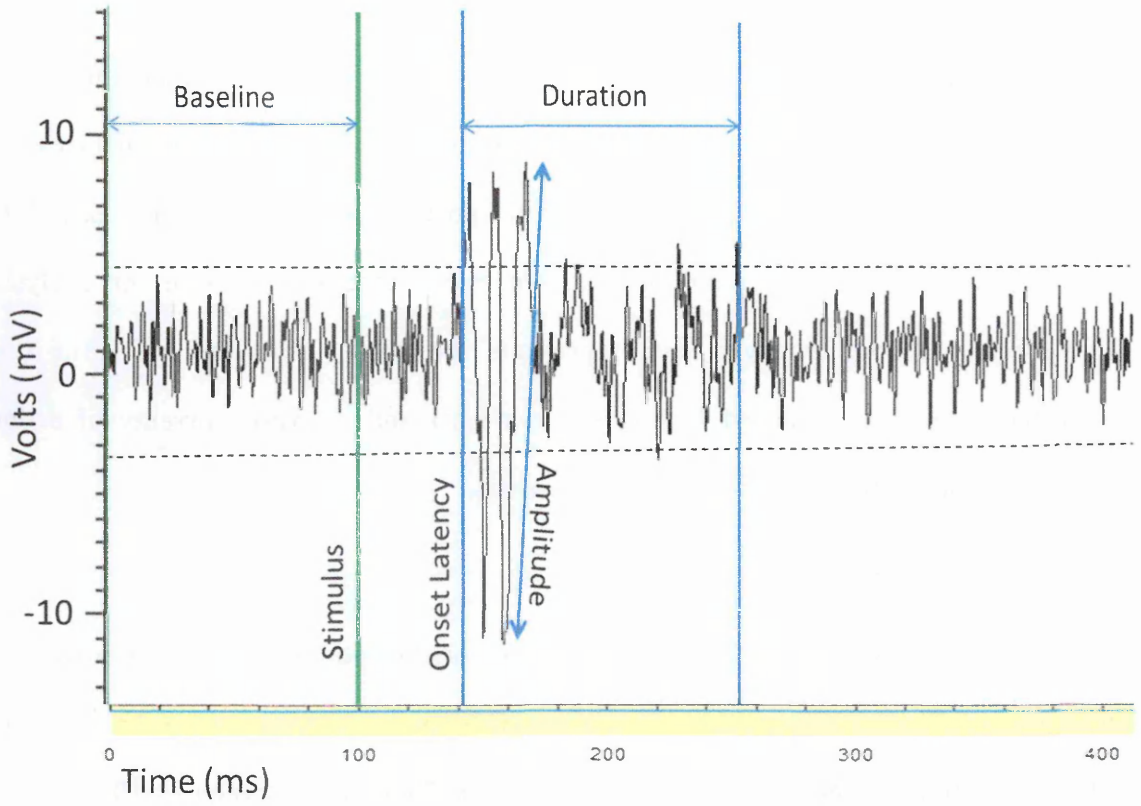

Figure 2.11: An LFP waveform average with the criteria for parameter measurements. The dotted lines indicate the threshold values with the lower line showing the baseline mean minus 1.96 standard deviations of that mean and the higher line showing the baseline mean plus 1.96 standard deviations of that mean.

Multiunit activity

A suitable threshold was used to extract unit activity from the raw trace, therefore removing background noise but keeping action potentials ('spikes') (see Figure 2.12 for an example). To prevent unit activity thresholds being affected by drug-induced changes, the threshold used to extract unit activity pre-drug was applied as a constant within each animal's dataset (i.e. the same threshold was used post-drug).

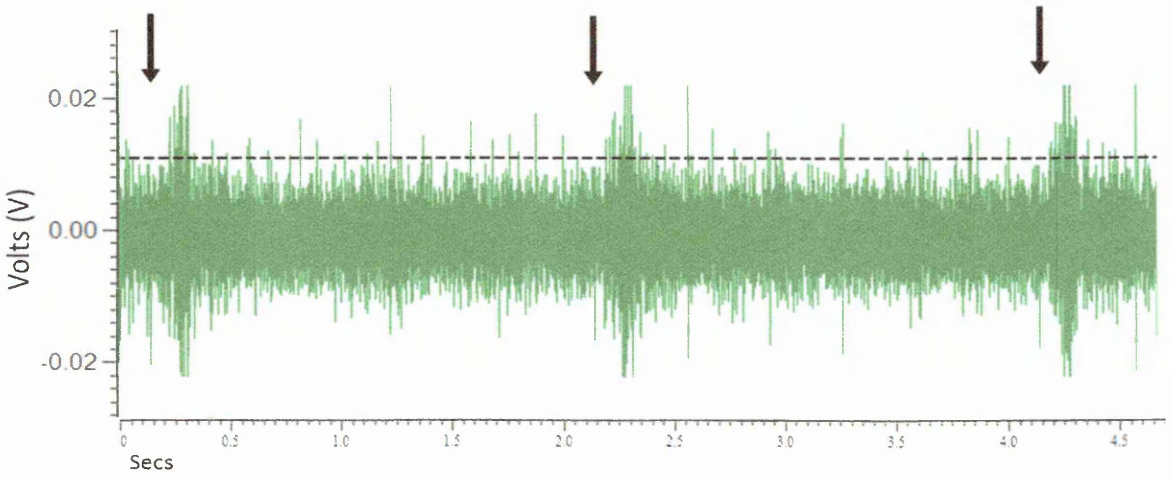

Figure 2.12: Example of a raw trace with the threshold set on the dotted line to extract spikes from the trace.

These spikes were then used to produce individual peri-stimulus time histograms (PSTH) in Spike2software for responses to 300 presentations of either the whole field light flash or the auditory tone. Each PSTH was 2 s in duration, with a bin width of 0.001 s extending from time T=-100 ms to +1900 ms, with the stimulus presentation at time T=0. The period between T=-100 and T=0 therefore served as a pre-stimulus baseline. The data from the PSTH was imported into a custom-made Excel macro (Peter Furness, Sheffield University, UK) for analysis of response parameters. As with the local field potential data, a response was deemed to have occurred if the trace extended beyond thresholds set at the mean ±1.96 standard deviations (SD) of the baseline activity. All responses were excitatory and therefore in reality this meant a response was deemed to have occurred if the level of activity rose above the upper threshold of the mean +1.96 SD, for at least 5 ms (5

consecutive bins), the first of which was labelled as the onset of a response. The duration was calculated by measuring when the response fell back to within the baseline levels for at least 10 ms (10 consecutive bins), the first of which was labelled as the end of the response. Duration was then given as the difference between onset latency and the response ending. The amplitude was recorded as the peak value of the response minus the mean baseline value (see Figure 2.13).

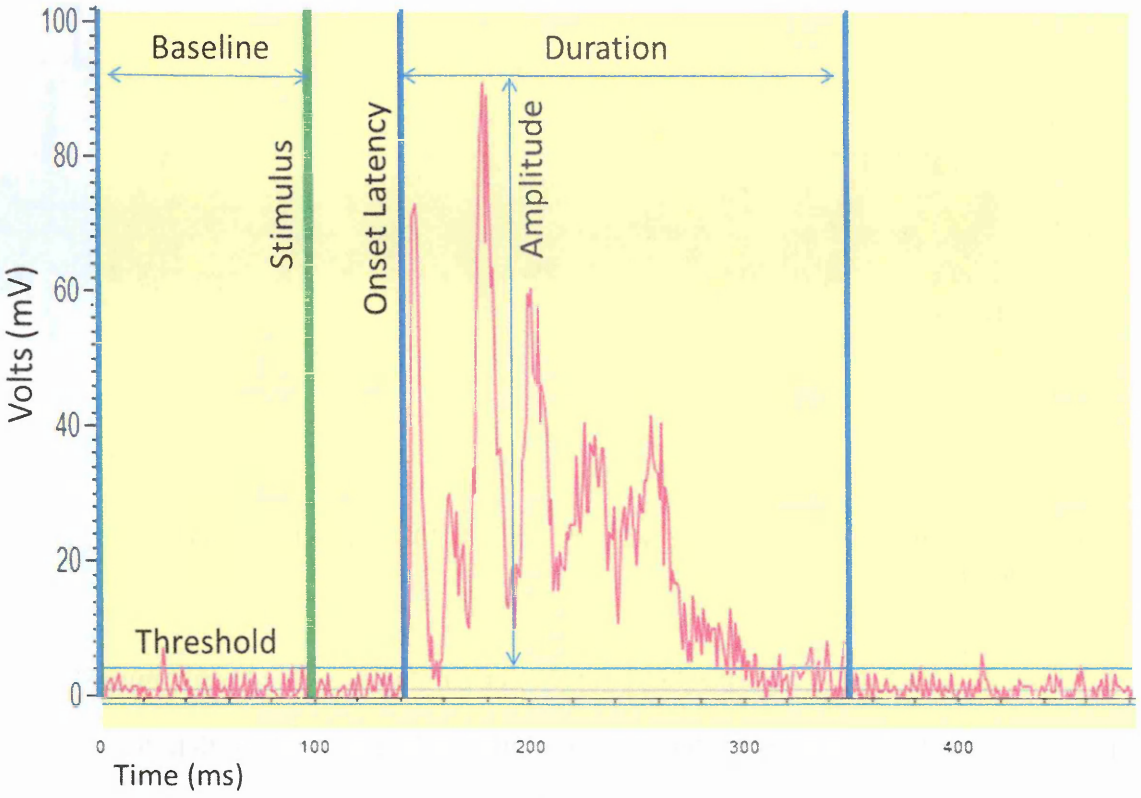

Figure 2.13: A multiunit activity PSTH converted in the macro with the criteria for parameter measurements.

All data (onset latency, amplitude and duration) from the local field potential and multiunit recordings were confirmed as having a normal distribution using the Kolmogorov–Smirnov test before analysis. The exact analysis used differed between experimental chapters depending on whether strain difference, stimulus intensity or drug dose was being investigated but in all cases statistical significance used a critical value of $P < 0.05$. Where the sphericity assumption was violated the Greenhouse Geisser correction was used.

2.4. POST-MORTEM PROCESSING

At the end of each electrophysiology experiment the recording sites in the superior colliculi were marked by passing direct current of 10 μA through the electrode for 5 seconds (Constant Voltage Isolated Stimulator DS2A MK2, Digitimer, UK). The deeply anaesthetised animal was then administered 1 ml of pentobarbitone (Animalcare, UK) prior to being transcardially perfused with 100 ml heparinised 0.9% saline, followed by 300ml 0.9% saline and subsequently fixed with 4% paraformaldehyde in phosphate buffer (pH 7.4). The brains were removed and placed in fixative overnight at 4 °C. Depending on the post mortem processing required techniques varied from this point and are explained below.

2.4.1. RECONSTRUCTION OF RECORDING SITES

For site reconstruction, the brains were removed from the fixative and placed in 20% sucrose for 36 hours before being frozen to -18 °C in isopentane (WWR International, UK) and cut into 50 μm coronal sections using a cryostat (CM1900, Leica, UK) with the cutting chamber held at -20 °C. The slices were then dehydrated with alcohol and Nissl stained with cresyl violet (0.5%) (Sigma Aldrich, UK), before cover-slipping for histological verification of recording sites (Figure 2.14) which were subsequently plotted onto reconstructed sections from Paxinos and Watson (1998) to confirm location of recording.

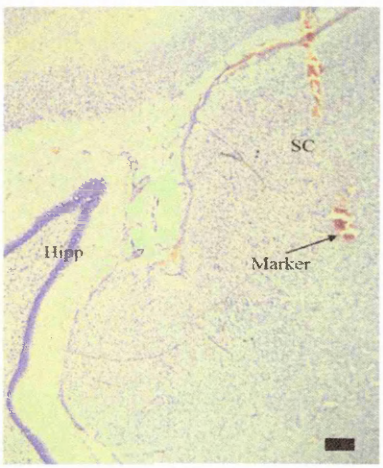

Figure 2.14: Marker identified by cresyl violet staining on a 50 μm brain slice. Measurements and identifiable brain markers were used to analyse where the recording was made. Hipp, Hippocampus; SC, superior colliculus. Scale bar = 100μm

2.4.2. VOLUME ANALYSIS

Brain volumes, as well as SC volumes were analysed using the Cavalieri principle of volume estimation; the principle states that if two solids have the same thickness and the same cross sectional area at every level, then they have the same volume (Figure 2.15; Howard and Reed, 1998). For volume analysis of both the whole brain and the superior colliculus, the brains were removed from the fixative and placed in 20% sucrose for 36 hours before being frozen to -18 °C in isopentane (WWR International, UK) and cut into 50 μm coronal sections using a cryostat (CM1900, Leica, UK) with the cutting chamber held at -20 °C. At a random starting point (between slices 1-5), every 5th section was collected for volume analysis. The slices were then dehydrated with alcohol and Nissl stained with cresyl violet (0.5%, Sigma Aldrich, UK) before cover-slipping for analysis using the Cavalieri principle of volume estimation.

Images were captured using a Microfibre digital camera attached to a Nikon Eclipse 80i microscope (Nikon UK LTD, Kingston-upon-Thames, UK) at a magnification of x1 (Nikon Plan UW, 1x/0.04, WD 3.2). The images, as well as an image of an appropriate scale bar were exported to a freely available reconstruction programme (Reconstruct version 1.1.0.1). By tracing the scale bar in the program, the dimensions in pixels per micrometer (μm) of the sections could be determined. The whole brain slice, as well as the superficial and deeper layers of the superior colliculus, as defined by Paxinos and Watson (1998) were then outlined throughout the slices using the Reconstruct programme. The multiplication of the cut surface area by the known distance in thickness (250 μm) was calculated to provide the estimated volume of the examined objects: the whole brain; superficial layers of SC; deeper layers of SC, or total SC.

To avoid bias, the first section was placed at a uniform and random position in a constant interval of length (t). Thus, an unbiased estimate of volume could be obtained by multiplying the total area of the sections through the structure on all the sections according to the formula below, where ΣA denotes the sum of section areas in μm^2 and t is the sectioning interval for the consecutive sections.

$$Volume = t \times \Sigma A$$

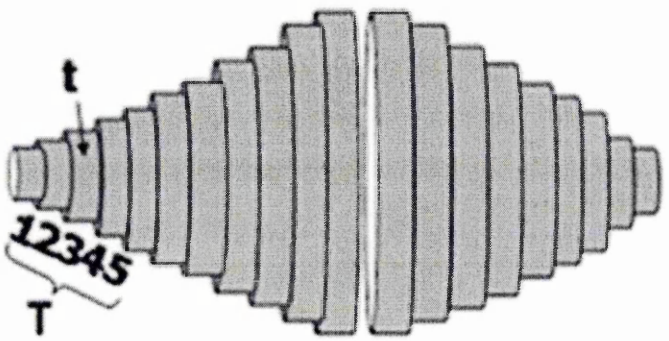

Figure 2.15: A schematic diagram of the measurement of volumes using Caverlieri principle. The estimated volume is the sum up of the area of every 5th section (t) multiplied by the constant distance (T). Adapted from Mandarim-de-Lacerda (2003).

Data were confirmed as having a normal distribution using the Kolmogorov–Smirnov test before analysis and volume results were then statistically analysed to investigate strain differences using a One-Way ANOVA. However, due to factors related to brain growth, such as the physical size of the animal, thought to influence the maximal size of an individual's brain (Raz et al., 1998; Sgouros et al., 1999), comparing solely volumes of intracranial structures between groups will not provide reliable data (Sgouros et al., 1999). Therefore, the volume fraction of the SC within the reference volume (the whole brain) was also calculated, to give a proportion of the structure (i.e. total SC, superficial layers, deeper layers) within the whole brain structure allowing for a coefficient of error (CE) value below 5% is within acceptable range (Gundersen and Jensen, 1987). The CE is a standard statistical value (standard deviation/mean) to predict the amount of sampling error (the

difference between an estimate and the true value), therefore evaluating the precision of stereological estimates.

$$Volume\ fraction = \frac{Volume\ of\ intracranial\ structure}{Volume\ of\ whole\ brain}$$

These data were also confirmed as having a normal distribution using the Kolmogorov–Smirnov test before analysis was conducted using a One-Way ANOVA to analyse strain differences.

2.4.3. CELL COUNTS

For cell counts within the superior colliculus, the brains were removed from the fixative and placed in 20% sucrose for 36 hours before being frozen to -18 °C in isopentane (WWR Internation, UK) and cut into 50 μm coronal sections using a cryostat (CM1900, Lecia, UK) with the cutting chamber held at -20 °C. At a random starting point (between slices 1-5), every 5th section was collected for volume analysis. The slices were then dehydrated with alcohol and Nissl stained with cresyl violet (0.5%, Sigma Aldrich, UK) before cover-slipping for analysis.

Images were digitalised using a Microfibre digital camera attached to a Nikon Eclipse 80i microscope (Nikon UK LTD, Kingston-upon-Thames, UK) with an ultrafine resolution motorised LEP X,Y stage and motorised z axis at a magnification of x40 (Nikon Plan Flor, 40x/0.75, DIC M, WD 0.72). Contours were drawn at low magnification (x1; Nikon Plan UW, 1x/0.04, WD 3.2) around the regions of interest as defined by Paxino and Watson (1998). The stereologically unbiased Optical Fractionator method on the Stereo-Investigator (version 7 software, MBF biosciences, Magdeburg, Germany) was used in real time to obtain an unbiased estimate of the total number of cells in the region of interest, as it is not influenced by the size, shape, spatial orientation, and spatial distribution of the cells studied.

The fractionator principle states that if you take as a random sample a known fraction of a population (West et al., 1991), then the unbiased estimate for the population is the value from the sample divided by the fraction; using the following formula.

$$Number\ of\ cells = \sum Q \times \frac{1}{Volume\ Analysed}$$

Where Q is the number of cell nuclei counted, and the volume fraction consists of the multiplication of three components; the section sampling fraction (ssf); area sampling fraction (asf) and height sampling fraction (hsf). By using the fractionator principle to select a series of systematically random sampled (SRS) sections and then sampling each section in the X, Y, and Z axis, again, by using the fractionator principle, total cell count estimates for the entire region of interest can be obtained. The ssf was 1/5, and was a constant as every 5th slice was examined; the starting section was taken randomly, between 1-5 to avoid bias.

$$ssf = \frac{1}{section\ interval}$$

Following this, the region of interest, being the superficial or deeper layers of the SC were manually traced on each slice; becoming the grid size area. A counting frame was used (see Figure 2.16) to count cells using the specific rules for counting cells in the frame (any cell within the frame or touching the top or left lines were included in the count; similarly any cell touching the bottom or right lines were excluded; if a cell touched top/left line and the bottom/right line; it was also excluded). The area sampling fraction can be measured using the formula below;

$$asf = \frac{counting\ frame\ area}{grid\ size\ area}$$

The grid spacing and counting frame size were X: 35 µm and Y: 35 µm and constant throughout the experiment for all animals. In a pilot (n=3) study, differing size counting frames and grids were used to identify the ideal parameters for the size and spacing of

counting frames, by taking in to account distribution of cells within and among sections as well as the heterogeneity of the distribution of cells within the SC, to allow for a sufficiently low CE (<0.05); and therefore an accurate estimate of cell counts. The location of the grid, and therefore counting frames were also randomly placed via stereo-investigator for each slice to avoid bias.

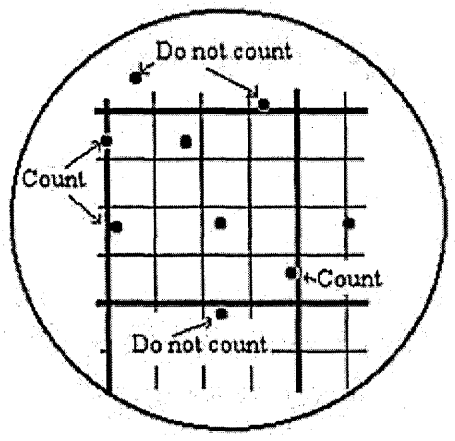

Figure 2.16: An example of a counting frame and inclusion criteria of cells (Williams and Radic, 1999)

As previously mentioned, the slices were cut at 50 μm. Due to shrinkage from alcohol dehydration, the thickness of the slice at each counting frame was measured (the distance between the point where the top of slice is in focus until the bottom of the slice is in focus). On average following shrinkage, the mean section thickness was 16.20±0.09 μm. Sufficient guard zones were set at 0.1 μm to remove any artefacts from the slicing process included in the counts. Therefore, the hsf can be worked out using the formula below:

$$hsf = \frac{dissector\ height}{average\ mounted\ section\ thickness}$$

When quantifying cells, the nucleus was chosen as the unambiguous location of a cell. A x40 magnification was used for a sufficiently small z-frame to allow for approximately 10 focal frames. A nucleus, and therefore cell, was only counted if the nucleus came into focus in the z-axis, then went out of focus to unambiguously identify the point within the dissector height, and thus the cell was counted.

In this investigation, nuclei from different cell types were differentiated based on morphological criteria of shape and relative size (see Figure 2.17). Neurons were identified by their generally larger cell bodies and non-spherical outline, as well as a pale and uniformly Nissl-stained cytoplasm with a well-marked nucleolus, often centrally located. Glial nuclei were identified by being generally smaller in size, ovoid shape with the absence of stained cytoplasm, the presence of a thicker nuclear membrane, and more heterogeneous chromatin within the nucleus (Sharma et al., 2005; Cotter et al., 2002).

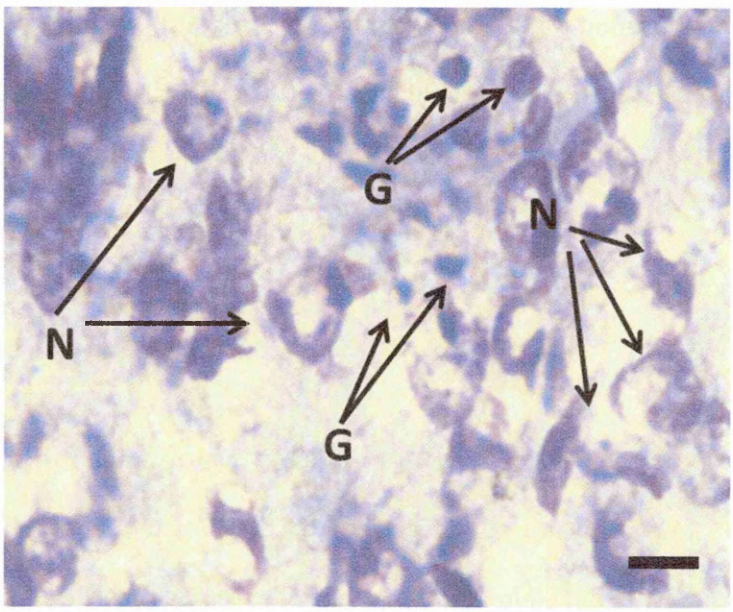

Figure 2.17: Examples of cresyl stained neuronal and glial cells in the SC at x40 magnification; differentiated based on specific morphological criteria. N: neuron; G: glia. Scale bar = 10μm.

2.4.4. IMMUNOHISTOCHEMSITRY

For immunohistochemistry techniques, brains were washed in 0.1 M phosphate buffer (PB), and sliced at 50 μm on a vibrating microtome (VT1000, Leica, UK) in 0.1 M PB. Every section through the superior colliculus was collected and placed in 0.1 M PB at 4 °C for 48 hours, with the solutions being replaced daily. Following this, all slices were placed in cryoprotectant (150 g sucrose, 150 ml Ethylene glycol, 200 ml 0.1 M PB) for 48 hours at 4 °C, with this solution also being replaced daily. The slices were subsequently stored at -

20 °C until immunohistochemistry was carried out. For all immunohistochemistry techniques the same experimental protocol was used, but the slices were incubated in a different primary antibody. All incubations were carried out at room temperature with agitation.

From each brain, four slices were collected for immunohistochemical analysis, at the same points throughout the SC, verified using structural markers, within the SC itself and surrounding structures such as the hippocampus (see Figure 2.18) at the following positions: anterior (around -5.80 mm relative to Bregma); medial (around -6.30 mm relative to Bregma); control (around -6.80 mm relative to Bregma); and posterior (around -7.30 mm relative to Bregma). The slices were inspected in real time using a Nikon Microscope (Nikon UK LTD, Kingston-upon-Thames, UK) to ensure they were cut at these specific points.

The sections chosen were clearly marked to identify which animal they came from, and then all placed in four separate pots according to the location of the slice, therefore all sections from each part of the SC for each animal were stained together.

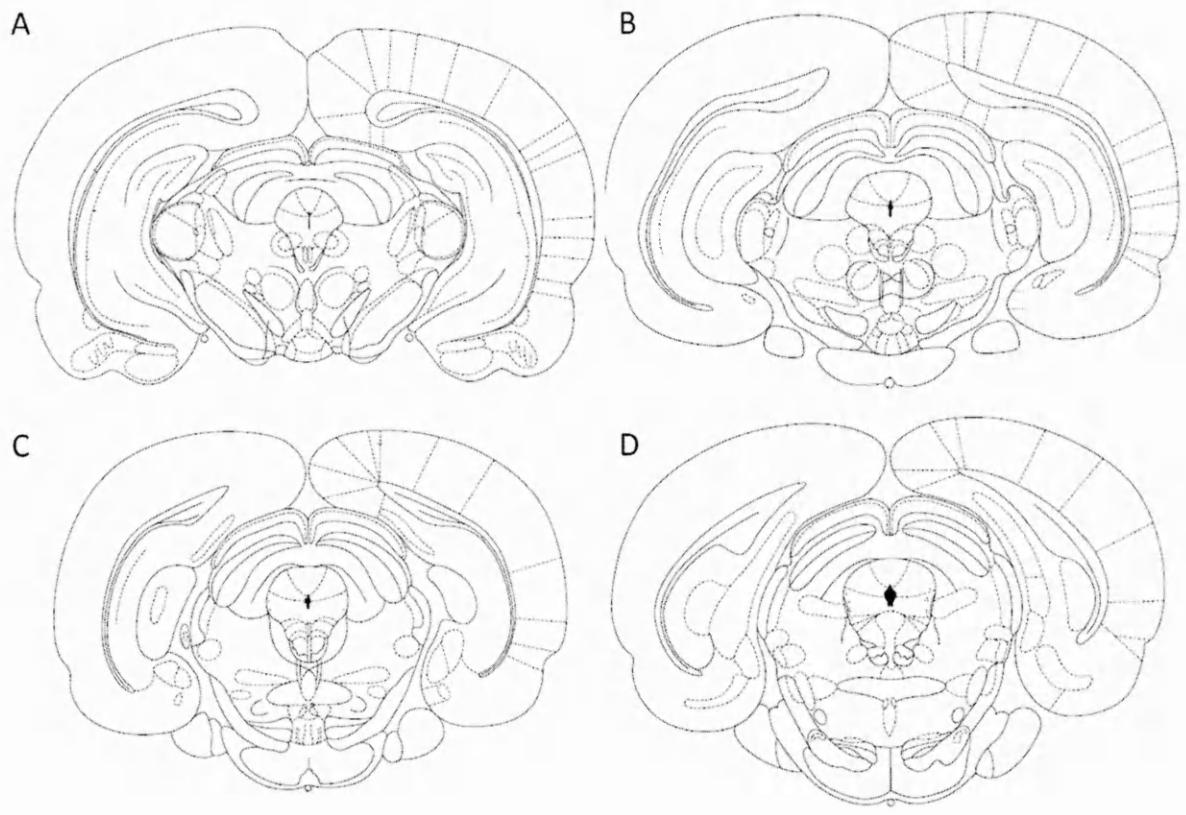

Figure 2.18: A series of 4 schematic images through the SC used for immunohistochemistry. A: -5.80 mm; B: -6.30 mm; C: -6.80 mm; D: -7.30 mm from Bregma. Adapted from Paxino and Watson (1998).

Free-floating sections were washed in 0.1 M PB (pH 7.4). To reduce aldehyde groups processing tissue, sections were incubated in 1% borohydride 0.1 M PB solution for 30 minutes, followed by another wash in 0.1 M PB. The tissues were then placed in 1% H_2O_2 for 20 minutes to block peroxidase, before being subsequently washed again in 0.1 M PB.

This was followed by 30 minutes of incubation in a blocking solution containing 0.01 M phosphate buffered saline (PBS, pH 7.4), 0.25% Triton-X100 (Sigma Aldrich, UK), 0.5% bovine serum albumin (BSA, Vector Laboratories Inc, USA) and 2% normal horse serum (NHS, Abcam, UK). The sections were then incubated for 24 hours at room temperature in the appropriate primary antibody solution. This consisted of the primary antibody for either 5-HT_{1AR} (Rabbit polyclonal antibody ab85615 1:500 dilution, Abcam, UK) or 5-HT_{1BR}

(Rabbit polyclonal antibody ab13896 1:500 dilution 1:200, Abcam, UK) diluted in an incubating buffer consisting of 0.01 M PBS, 2% NHS, 0.1% BSA and 0.25% Triton-X100.

The following day, the sections were subsequently rinsed in 0.01 M PBS and then incubated for 2.5 hours in the secondary antibody biotinylated anti-rabbit IgG made in horse (dilution 1:200, W0614, Vector Laboratories, UK) diluted in 0.1% BSA, 0.1 M PBS. The sections were then washed in 0.1M TS (Tris Buffered Saline; Sigma Aldrich, UK) and incubated in Vector elite ABC solution reagent (Vector Labs, California, USA; 1:50 in 0.1% BSA in 0.1M TS) in 0.1% BSA in 0.1M TS for 30 minutes. Following subsequent washes in 0.1M TS the immunoreactivity was revealed with exposure to diaminobenzidine (DAB) (0.35 mg/ml) with H_2O_2 in 0.1M TS for 5 minutes. A sample section was examined under the microscope in order to check the exposure time had been sufficient. If sufficient, the reaction was stopped by washing in 0.1M TS, followed by 0.1M PB. Slices were then mounted onto gelled electrolyte slides, and left to dry for 24 hours before being dehydrated through alcohol and cleared in xylene (WWR Internation, UK) before being cover-slipped with DPX (WWR Internation, UK).

Images were digitalised using a Retiga 2000R (Qimaging) digital camera attached to a Nikon Microphot-FX microscope (Nikon UK LTD, Kingston-upon-Thames, UK) at x4 magnification(Nikon Plan Flor 4x/0.13). Contours were drawn at low magnification (x4; Nikon Plan Flor 4x/0.13) around the regions of interest as defined by Paxino and Watson (1998).

For each marker the percentage of immunoreactivity per area of the regions of interest was analysed in Image-Pro Plus (version 5, Media Cybernetics, Marlow, UK) by analysing the optical density of the immunoreactivity (see Figure 2.19). Lamp intensity, digital camera set

up and microscope calibration were kept constant throughout the image collection. The multi-colour images were split into red, green and blue single channels; to allow image analysis to occur on the single red channel only. The foreground immunoreactivty was accurately defined for each marker and threshold of immunoreactivty set manually by choosing an appropriate threshold that selected the foreground immunoreactivty above background. All analysis was done blind to strain of animal. This setting was then applied across all images giving a consistent intensity data between animals; the average percentage of immunoreactivity per region of interest per animal was then established.

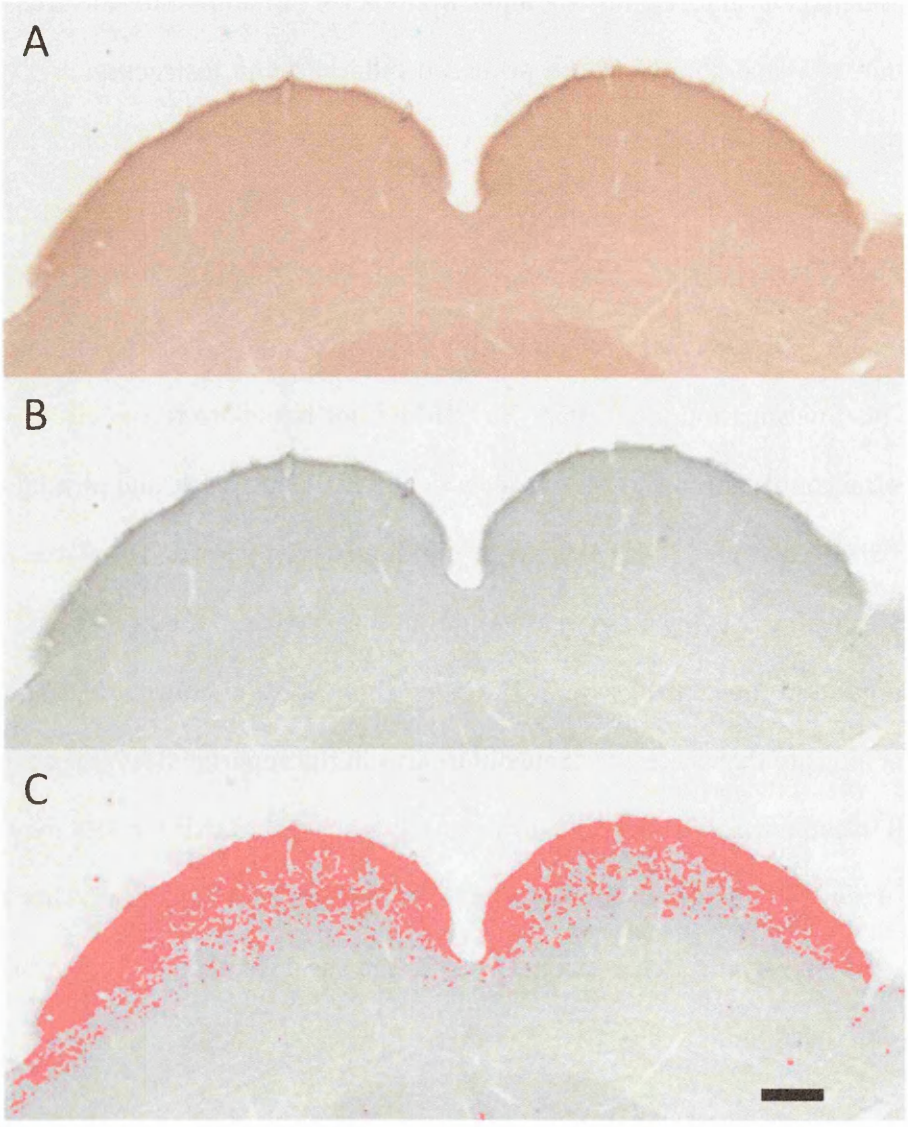

Figure 2.19: Immunoreactivity: A: original image taken; B: single channel image; C: threshold applied. Scale bar = 200μm

3. COLLICULAR VISUAL RESPONSES

This chapter describes the findings from an investigation into visual processing in the superficial layers of the SC of the SHR model of ADHD using behavioural, physiological and morphological analyses. As suggested in the Introduction (Chapter 1), alterations within the SC could underlie ADHD and therefore some of the ADHD-like behaviours seen in the SHR. The key methods for this chapter are detailed in Material and Methods (Chapter 2). It has been hypothesised (see Section 1.4) that the SHR will show differences in responsiveness in comparison to the two control strains towards the visual distraction stimulus in an SC-dependent behavioural task, and will differ in the level of habituation towards a repeated presentation of visual stimuli. These proposed differences in distraction and habituation are hypothesised to be due to enhanced activity in the SC. Therefore, from a physiological stand point, it is hypothesised that the SHR will show differences in superior colliculus superficial layer responses to a visual stimulus. Finally, it is hypothesised that differences in physiological responses may be accounted for by morphological differences in neuronal cell counts. This investigation found that the SHR did not habituate to the visual stimulus as quickly as the control strains in the SC-dependent behavioural task, and physiologically the SC of the SHR was more likely to have a multiunit activity response at the lower light intensities as well as having an increased multiunit response to the higher light intensities when compared to the control strains. However, there were no morphological differences. These data indicate that there are some differences in the superficial layers of the colliculus in the SHR in comparison to two control strains. It is suggested that the SHR may perceive a heightened saliency of stimuli in comparison to the two control strains. This heightened collicular response would give a 'stronger bid' to the basal ganglia, increasing the chance of a response to the stimuli.

3.1. INTRODUCTION

The inability to inhibit unnecessary behavioural responses or habituate to stimuli that are neither salient nor novel is found in individuals with ADHD (Quay, 1997; Dimoska et al., 2003) and in the SHR (Li et al., 2007; Sagvolden et al., 1993). Similarly, behavioural inhibition impairment is a central unifying theory of ADHD (Barkley, 1997; Nigg, 2001; Sergeant et al., 2002), whereby the ability to inhibit unnecessary or inappropriate behavioural responses is lowered in individuals with ADHD. This can lead to core symptoms such as impulsivity (Quay, 1997). Intolerance to delayed reinforcement is also thought to underlie ADHD (Logue, 1988), yet a greater preference for immediate gratification may be linked to a behavioural inhibition impairment where the child has difficulty withholding a response. Both behavioural inhibition and habituation can be linked to the SC. The circuitry in the SC integrates information from the frontoparietal network with the assessment of the physical salience of stimuli, to produce a retinotopic depiction of the relative importance of locations as the subsequent locus for the orientation of attention and gaze (Fecteau and Munoz, 2006; Dorris et al., 2007; Shen and Pare, 2007; Mysore et al., 2011). Therefore, any dysfunction in the processing of saliency of stimuli within the SC will cause deficits in behavioural inhibition and habituation, which could result in the symptoms of ADHD.

As previously mentioned in Chapter 1, in mammals, the superficial layers of SC have the functional ability to decrease thresholds and augment response gains and resolution in the retinotopically organised visual forebrain areas (Luck et al., 1997; Reynolds and Chelazzi, 2004; Shipp, 2004; Maunsell and Treue, 2006) via thalamic nuclei (Reiner and Karten, 1982; Boehnke and Munoz, 2008). Bilateral removal of the SC causes rats to show no orienting reflex or distraction to the presentation of novel visual or auditory stimuli. However visual-decorticated rats do show various components of the orienting reflex or disturbance in task performance when the same novel stimuli are presented (Goodale and

Murison, 1975). Therefore, the SC mediates the shift in visual fixation and attention, while the visual cortex contributes to visuospatial guidance of locomotor movements, but does not play a significant role in the control and integration of the orienting reflex (Goodale and Murison, 1975). Interestingly, the SC-lesioned animals are also hyperactive; another symptom of ADHD (Weldon and Smith, 1979). Similarly, an enhanced and sustained response occurs in the monkey if a selected visual stimulus as the target for a shift in gaze is found in a superficial neuron's receptive field (Wurtz and Mohler, 1976; Li and Basso, 2005; 2008). Also, the disconnection of the SC from the controlling influences of the prefrontal cortex leads to an increase in distractibility in humans (Gaymard et al., 2003) and a decrease in distractibility has also been observed in SC-lesioned animals in an array of species (cat: Sprague and Meikle, 1965; rat: Goodale et al., 1978; monkey: Milner et al., 1978).

A dopaminergic projection may also be critical to reinstatement of visual orienting behaviour adversely affected by SC lesions. The SC has an efferent pathway to the dopaminergic cell bodies in the substantia nigra pars compacta (Comoil et al., 2003). Damage to this pathway will disturb processing of sensory information necessary for orienting behaviour by impairing a mechanism that provides a tonic background as well as affecting the phasic activity of the nigrostriatal processing of sensory information (Dommett et al., 2005; Coizet et al., 2006), which furthermore will cause a lack of behavioural inhibition and habituation to non-salient stimuli behaviourally (see Section 1.3.2). As previously mentioned in Section 1.3.2.2, dysfunction of dopaminergic transmission in SHRs has been implicated in the impairment of attention and hyperactivity reported in these animals (Puumala and Sirvio, 1998). Similarly, dysfunction of the dopamine system is an underlying theory in ADHD (see Section 1.1.6).

In light of this evidence an SC-dependent behavioural task was used to assess the SHRs habituation to a repeated visual stimulus. It is hypothesised that the SHR will show deficits in the habituation to the visual stimuli due to a dysfunction in the processing of the saliency of the stimuli. Furthermore, physiological and morphological differences will be found in the superficial layers of the SC in the SHR. Specifically, it is hypothesised that physiological stimulus responses in the SC will be different in the SHR, leading to secondary effects in the saliency of stimuli, and the ADHD-like distractibility behaviours seen in this strain. Morphological differences in neuronal cell counts are also hypothesised to be the cause of these physiological differences.

Hypotheses

- There will be behavioural differences in how the SHR responds and habituates to a visual stimulus in an SC-dependent task in comparison to the two control strains (WKY and WIS).
- There will be physiological differences in the responses to visual stimuli recorded in the superficial layers of the SC in the SHR in comparison to the two control strains (WKY and WIS).
- There will be morphological differences in the superficial layers of the SC in the SHR in comparison to the two control strains (WKY and WIS).

3.2. METHODS

A total of 110 rats were used for the experiments described (SHR N=35; WIS N= 38; WKY N=37). Following the behavioural experiments, the animals were used for the electrophysiological experiment, but the animals used for the morphological experiments were singularly used for this alone. The weight of the animals immediately prior to experimentation is detailed, by strain and experiment, in Table 3.1. The normality of the weight data was confirmed using the Kolmogorov–Smirnov test and a One-Way ANOVA was conducted to examine where there were any strain differences in weight for each type of

experiment. This revealed a significant difference in weight between the strains for the behavioural experiment (F=8.39; df=2; p=0.002). Post hoc (Tukey HSD) tests revealed the WIS had a significantly greater weight than the WKY (p=0.002) and the SHR (p=0.026), but that there was no significant difference between the WKY and SHR (p=0.554). Similar findings were seen in the electrophysiological experiment (F=28.19; df=2; p=0.0005). Post hoc (Tukey HSD) tests again showed the WIS had a significantly greater weight than the WKY (p=0.0005) and the SHR (p=0.0005), but that there was no significant difference between the WKY and SHR (p=0.927). There was no significant difference in weight between the strains for the cell count experiment (F=1.33; df=2; p=0.311) and volume experiment (F=3.14; df=2; p=0.092).

Experiment		SHR	WIS	WKY
Behaviour task	Number of subjects	9	8	9
	Mean weight ± SEM (g)	399.97±11.17	448.25±10.08	466.34±14.07
Electrophysiology	Number of subjects	27	30	29
	Mean weight ± SEM (g)	396.43±6.26	489.75±12.55	401.71±9.42
Volume Estimation	Number of subjects	4	4	4
	Mean weight ± SEM (g)	396.78±16.24	484.83±28.71	431.00±28.14
Cell counts	Number of subjects	4	4	4
	Mean weight ± SEM (g)	413.85±16.27	455.55±19.47	410.45±27.84

Table 3.1: The mean ±SEM weights and number of subjects for the experiments within this chapter.

In order to analyse strain differences and the effect of increasing intensity of a visual stimulus on collicular responses using the measured parameters (onset latency, amplitude, duration), repeated measures ANOVA was used with STIMULUS INTENSITY as the within-subjects factor and STRAIN as the between-subjects factor. All data were confirmed as having a normal distribution using the Kolmogorov–Smirnov test before being analysed.

3.3. RESULTS

3.3.1. BEHAVIOUR

Level of responding and habituation

Responsiveness towards the distractor stimulus was defined in Section 2.2.1 of Chapter 2 as if the animal froze in response to the stimulus, physically interacted with the stimulus or oriented its head towards the stimulus. Using this definition of responding, all SHR and WKY and 87.5% (7/8) WIS responded towards the visual stimulus on the first presentation. Overall, this high level of responding is to be expected because the stimulus is novel. Therefore, we examined how the level of responding changed with each consecutive presentation of the stimulus by plotting the percentage of animals responding to the stimulus as a function of stimulus presentation. Figure 3.1a shows that the percentage of animals responding to the stimulus decreases with increasing number of stimulus presentations for all three strains. However, the rate at which the responding decreases differs by strain with 66.67% of SHR still responding to the stimulus by the 10th trial in comparison to just 11.11% of WKY and no WIS. A survival analysis life table was used to examine the differences between the three strains in terms of this drop in responsiveness, or put another way, the habituation to the stimulus over the consecutive stimulus presentations, where TIME was the 5 second epoch of each trial (10 trials), STATUS was whether the animal habituated to the stimulus (1= habituated, 0=responded), and the FACTOR was STRAIN. The median survival time is the time at which 50% of those who originally started out responding have habituated and so no longer respond. The SHR had a median survival time of 10.00, WIS had a median survival time of 8.31 and WKY had a median survival time 8.37. There was a significant difference in STRAIN ($F=28.96$; $df=2$; $p=0.0005$). Post-hoc analysis revealed that the SHR was significantly more likely to persistently respond to the light stimulus in comparison to the WIS ($F=26.39$; $df=1$; $p=0.0005$) and WKY ($F=27.33$; $df=1$; $p=0.0005$). There was no significant difference between the WIS and WKY ($F=0.044$; $df=1$; $p=0.835$).

In addition to looking at the period for which the stimulus was on, the pre- and post-5 second epoch was also examined to ensure the differences found were not due to general behaviour towards the object in the arena. Figure 3.1 b and c shows that the level of engagement with the stimulus was lower in the pre and post stimulus ON periods than when the stimulus was ON as may be expected. However, there was no decline in the pre- and post- stimulus epochs, indicating that the decline in engagement while the stimulus was on was not due to the animals' behaviour in the arena but was specifically due to stimulus ON interaction.

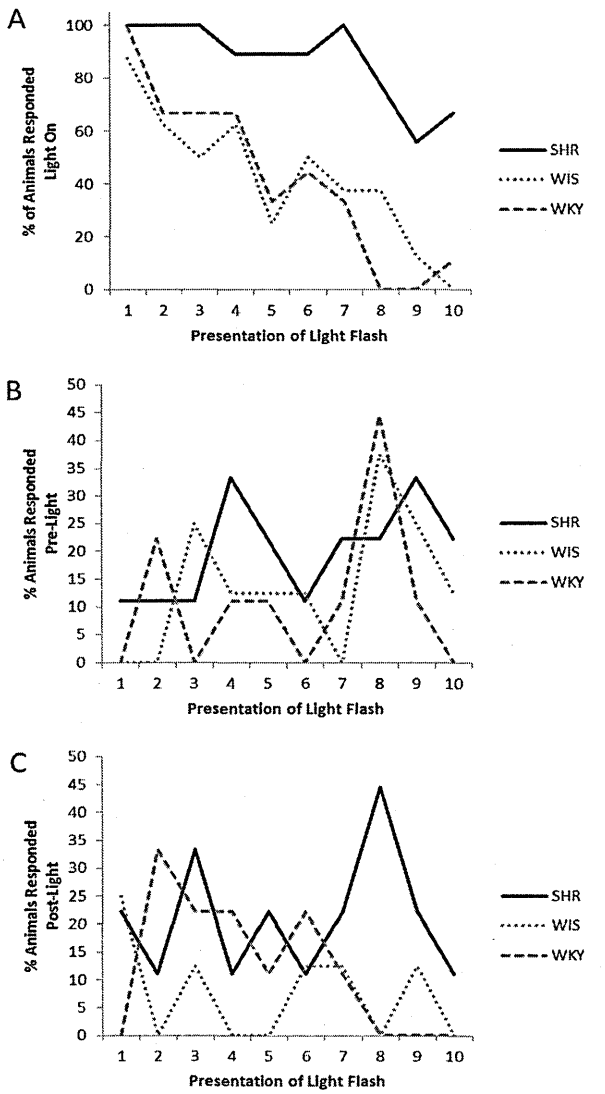

Figure **Error! No text of specified style in document.**.1: The percentage of animals responding to the light equipment over the 10 consecutive trials: A: 5-seconds during stimulus; B: 5-seconds pre-stimulus C: 5-seconds post-stimulus. The SHR persistently responded to the light flash over all 10 trials in comparison to the two control strains.

Duration of response

During the 5 second time period when the light stimulus was on all three strains spent a similar amount of time engaged with the stimulus during the first presentation (SHR: 57.56±8.97% of total time, or 2.88±0.45 s, WIS: 52.50±9.13% of total time; or 2.63±0.48 s; WKY: 54.89±6.19% of total time, or 2.74±0.31 s (see Figure 3.2a). Repeated measures ANOVA with STIMULUS PRESENTATION as the within-subjects factor and STRAIN as the between-subjects factor was conducted using the percentage of overall time distracted by the stimulus as the dependent variable. There was a significant main effect of STIMULUS PRESENTATION (F=14.80; df=5.67, 0.39; p=0.0005). All animals spent significantly less time responding to the stimulus as the consecutive trials occurred, with significant time decreases compared to the 1st stimulus beginning at the 2nd stimulus (F=5.45; df=1, 0.19; p=0.029). By the final stimulus there was a highly significant time difference in the duration of their response (F=63.39; df=1, 0.73; p=0.0005). There was also a significant main effect of STRAIN (F=31.93; df=2, 0.74; p=0.0005), with post hoc (Tukey HSD) tests showing that the SHR spent significantly more time responding to the light stimulus than the WIS (p=0.0005) and the WKY (p=0.0005). There was no significant STIMULUS PRESENTATION x STRAIN interaction (F=0.83; df=11.33; 0.67; p=0.067).

During the 5 seconds prior to the onset of the light stimulus, the animals showed very little interest in the stimulus equipment (See Figure 3.2b), spending only brief periods responding to it (SHR 7.33±7.33% of total time or 0.37±0.37 s; WIS 0.00±0.00% of total time or 0.00±0.00 s; WKY 0.00±0.00% of total time or 0.00±0.00 s) as shown in Figure 3.2. Repeated measures ANOVA with STIMULUS PRESENTATION as the within-subjects factor and STRAIN as the between-subjects factor was conducted using the percentage of overall time responding to the stimulus as the dependent variable. There were no significant main effects of STIMULUS PRESENTATION (F=1.149; df=2.84, 0.048; p=0.335) or STRAIN

(F=1.68; df=2, 0.128; p=0.208). There was no significant STIMULUS PRESENTATION x STRAIN interaction (F=0.87; df=5.68, 0.07; p=0.518).

The behaviour in the 5 second post-stimulus was also analysed (See Figure 3.2c). Similarly to the pre-stimulus time period, all strains spent very little time engaging with the stimulus equipment during this time. When averaged across all trials, the SHR, WIS and WKY spent 7.11±6.17% (0.36±0.31 s), 4.50±2.97% (0.23±0.15 s), and 0.00±0.00% (0.00±0.00 s) respectively responding to the stimulus (see Figure 3.2). Similarly, repeated-measures ANOVA with STIMULUS PRESENTATION as the within-subjects factor and STRAIN as the between-subjects factor was conducted using the percentage of overall time distracted by the stimulus as the dependent variable. There were no significant main effects of STIMULUS PRESENTATION (F=0.81; df=3.16; 0.034; p=0.496; see Figure 3.2) or STRAIN (F=0.79; df=2, 0.064; p=0.468). There was no significant STIMULUS PRESENTATION x STRAIN interaction (F=1.33; df=6.31, 0.104; p=0.253).

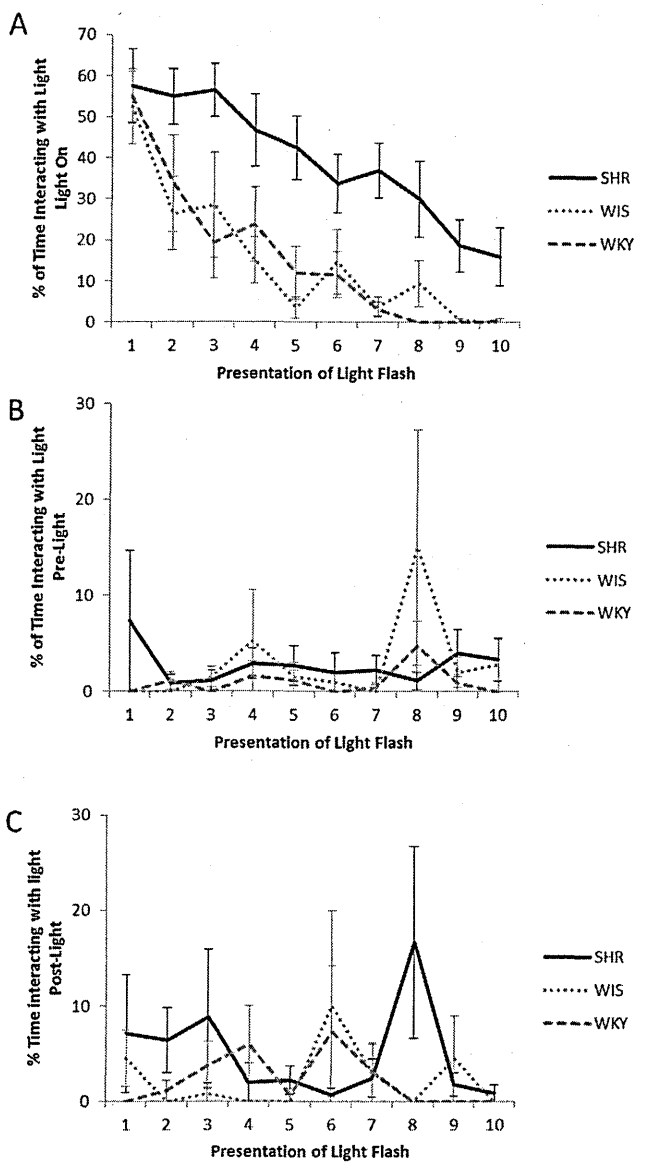

Figure 3.2: The mean ± SEM percentage of time spent engaging with the light equipment over the 10 consecutive trials: A: 5-second pre-stimulus; B: 5-seconds while light was on; C: 5-seconds post-stimulus. There were no significant main effects of reoccurring stimulus presentation, strain or interactions for pre- and post-stimulus, yet there was a significant main effect of strain, with the SHR spending significantly more time with the stimulus while the light was on.

In summary, behavioural testing revealed that the SHR responded to the stimulus more frequently and for a longer duration, showing reduced habituation in comparison to the two control strains. There were no differences between strains in responsiveness towards the stimulus object in the absence of the stimulus itself, suggesting this is specific to the sensory event.

3.3.2. PHYSIOLOGICAL DIFFERENCES

Inclusion criteria

The recording positions of the 86 visual responses (27 SHR; 30 WIS; 29 WKY) used in the data analysis were all in the superficial layers of the SC, as shown in the reconstruction of the sections in Figure 3.3 and tabulated in Table 3.2 and Table 3.3. Of the 86 visual recordings used to compare responses to different visual stimulus intensities, 32 were recorded in Opticum (Op), (12 SHR; 10 WIS; 10 WKY), 53 were recorded in Superficial Grey (SuG) (15 SHR; 20 WIS; 18 WKY) and 1 was recorded from Zonal Layer (Zo) (WKY). Chi-square analysis showed there were no significant association between strain and recording position in anterior-posterior positioning (χ^2=1.71; df=4; p=0.789), medial-lateral positioning (χ^2=0.29; df=2; p=0.867) or superficial layer (χ^2=2.81; df=4; p=0.590).

Co-ordinates From Bregma	Layer	SHR N=27	WIS N=30	WKY N=29
-5.8mm	Zonal Layer	0	0	0
	Superficial Grey	2	5	4
	Opticum	2	2	2
-6.3mm	Zonal Layer	0	0	1
	Superficial Grey	9	11	11
	Opticum	6	7	5
-6.8mm	Zonal Layer	0	0	0
	Superficial Grey	4	4	3
	Opticum	4	1	3

Table 3.2: The anterior-posterior and layer positioning of the electrodes for the visual responses within the superficial layers of the superior colliculus for each strain. Chi-square analysis revealed no significant association.

	SHR	WIS	WKY
Medial Recordings	17	19	20
Lateral Recordings	10	11	9

Table 3.3: The medial-lateral positioning of the electrodes for the visual responses. Chi-square analysis revealed no significant association.

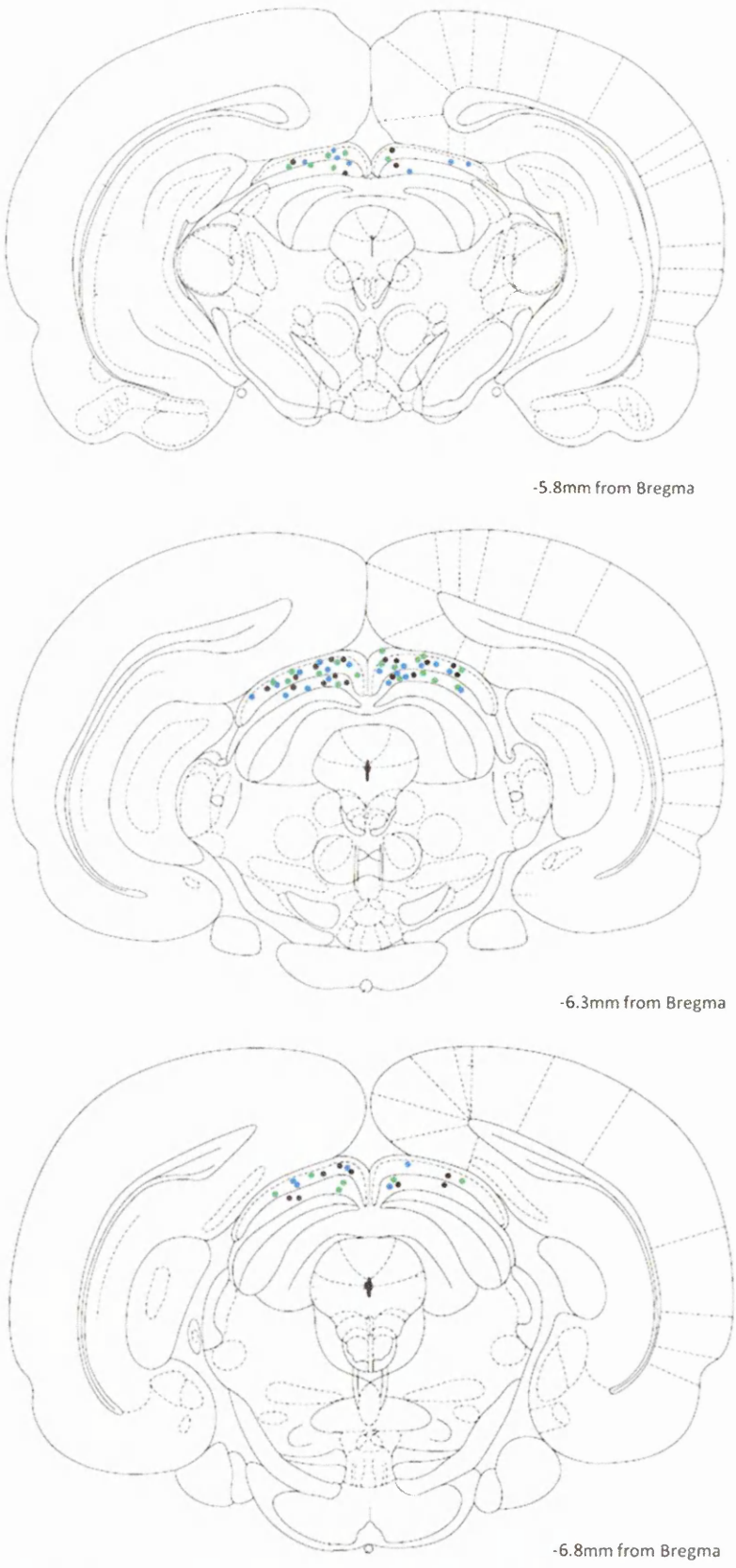

Figure 3.3: Reconstructed plots of recording sites in the SC. During collicular recordings, SHR recording sites are shown in black, WKY recording sites are shown in green, and Wistar recording sites are shown in blue. Adapted from Paxinos and Watson (1998).

3.3.2.1. VISUAL STIMULATION LOCAL FIELD POTENTIALS

Stimulus- response relationship

Responses were recorded to five different intensities of a light stimulus as outlined in Section 2.3.4, an example of a visual LFP waveform average at the middle intensity of a stimulus-response curve for each strain is shown in Figure 3.4. A response was deemed to have occurred if the trace exceeded beyond a pre-determined threshold after stimulus onset, according to criteria in Section 2.3.5. Based on these criteria not all animals responded to all stimulus intensities.

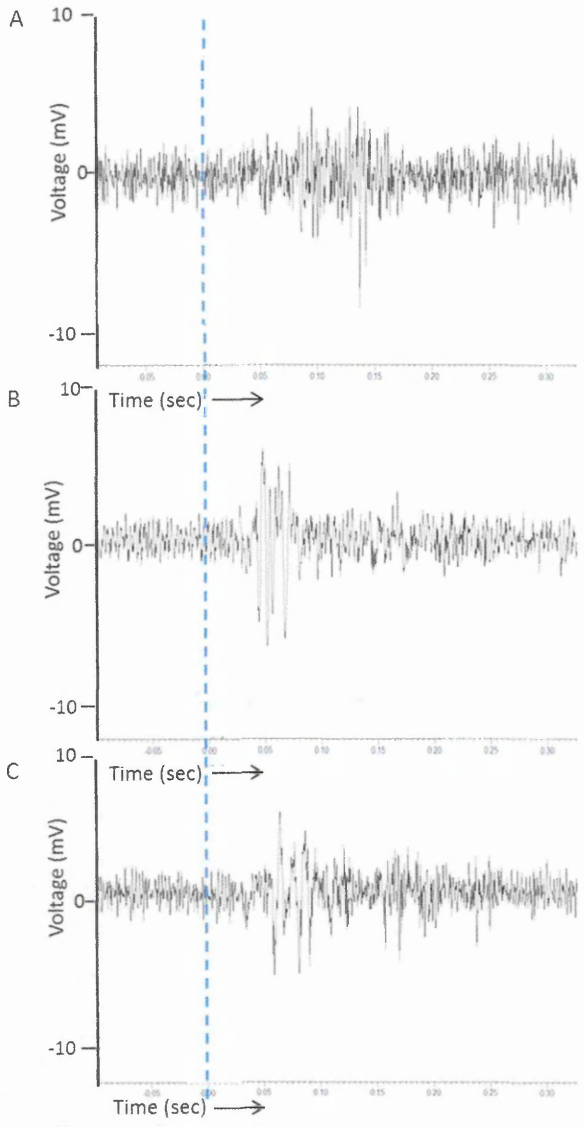

Figure 3.4: An example of a visual response LFP waveform average at the middle intensity of a stimulus-response curve: A: SHR; B: WIS; C: WKY. The dotted blue line represents the point in time when the stimulus occurred.

The percentage of animals responding at each intensity is shown by strain in Table 3.4 and Figure 3.5, where stimulus intensity 4 Mcd is the lowest intensity and stimulus intensity 20 Mcd is the highest. Animals were least likely to respond to the 4 and 8 Mcd stimulus where maximum responsiveness was 70.4 % and 75.9 % respectively. A Chi-Square analysis revealed no significant association between strain and likelihood of responding at the first or second intensity (see Table 3.4 for statistics). By the 12 Mcd intensity the majority of animals were responding and again there were no differences in likelihood of responses between the strains.

Stimulus intensity (Mcd)	Percentage of animals responding			Analysis of strain differences		
	SHR(n=27)	WIS (n=30)	WKY(n=29)	χ^2	df	p
4	70.4	50.0	48.4	5.05	2	0.282
8	75.9	73.3	61.3	1.75	2	0.416
12	92.6	76.7	79.3	2.82	2	0.244
16	100	96.7	96.6	0.99	2	0.626
20	100	100	100	N/A		

Table 3.4: The percentage of rats that responded at each light intensity (Mcd). There were no significant differences in strain.

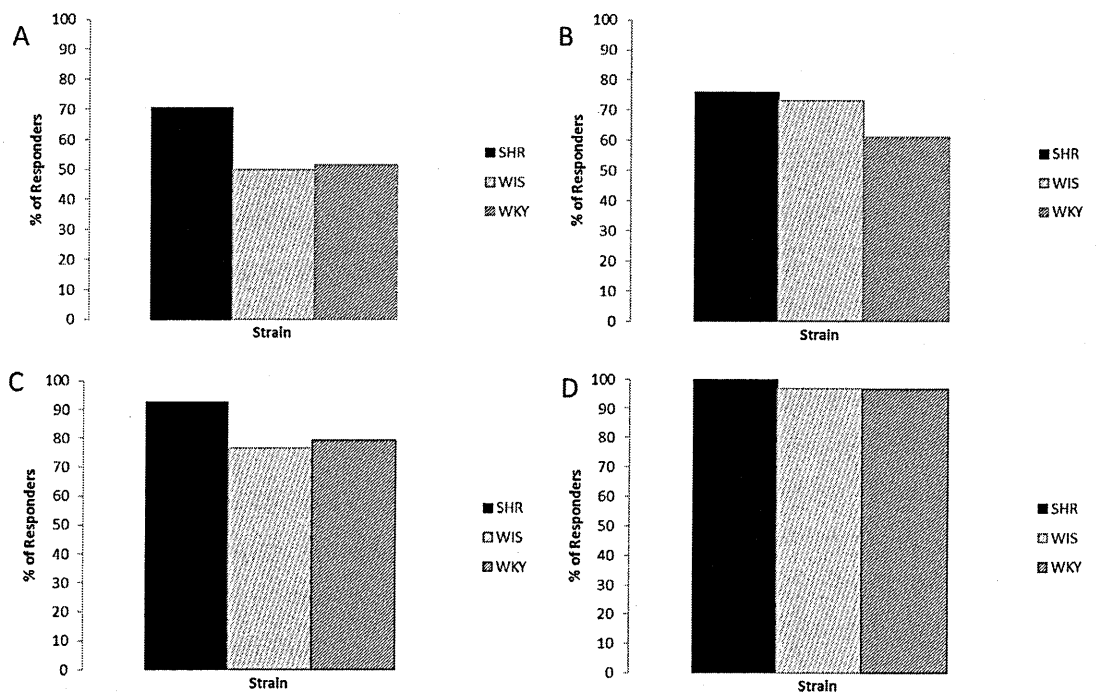

Figure 3.5: The percentage of rats that produced a LFP light response to the lowest four light levels: A: 4 Mcd; B: 8 Mcd; C: 12 Mcd; D: 16 Mcd. All animals responded to the final intensity of 20 Mcd (data not shown). There were no significant differences found in LFP responsiveness.

In order to look at effects of stimulus intensity, data from only the animals that responded to the final three intensities were analysed (12, 16 and 20 Mcd), and hence only data from animals that responded to the final three intensities were used (parameters: onset latency; peak-to-peak amplitude; duration; final N: SHR n=25; WKY n=23; WIS n=23). All parameters were analysed using repeated measures ANOVA with STIMULUS INTENSITY as the within-subjects factor and STRAIN as the between-subjects factor.

Onset latency

The onset latency for visual responses to stimulus intensities of 12-20 Mcd is shown in Figure 3.6 as the mean ± SEM. There was a significant main effect of STIMULUS INTENSITY (F=27.62; df=1.73, 0.92; p=0.0005). Within-subject contrasts showed that there was a significant decrease in onset latency between the 12 Mcd and 16 Mcd stimulus intensity (F=10.09; df=1, 0.13; p=0.002), the 12 Mcd and 20 Mcd stimulus intensity (F=44.88; df=1, 0.40; p=0.0005) and 16 Mcd and 20 Mcd stimulus intensity (F=26.34; df=1; p=0.0005). There was no significant main effect of STRAIN (F=0.32; df=2, 0.01; p=0.730). There was no significant STIMULUS INTENSITY x STRAIN interaction (F=2.23; df=3.46, 0.61; p=0.080).

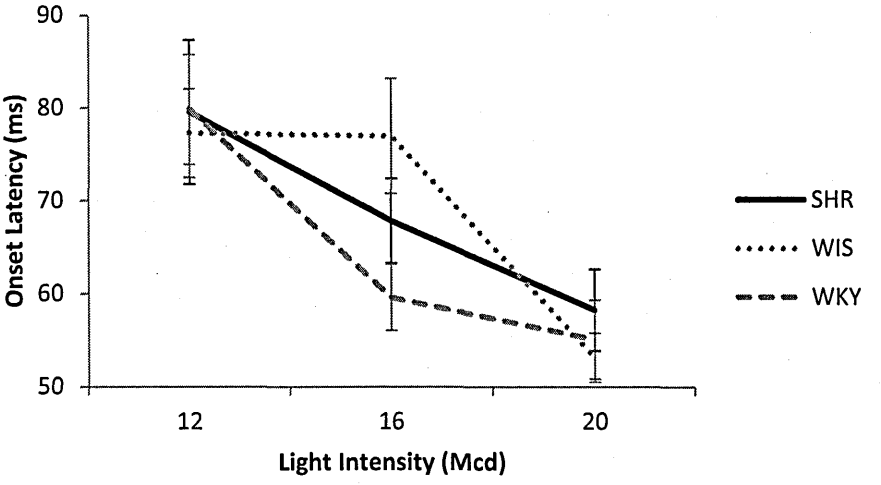

Figure 3.6: The mean ± SEM LFP visual response onset latency of the three strains with increasing stimulus intensities showing a significant main effect of stimulus intensity, with decline in onset latency as the intensity increased. There were no significant main effects of strain or interactions between strain and light intensity.

Peak-to-peak amplitude

The peak-to-peak amplitude for responses to stimulus intensities of 12-20 Mcd is shown in Figure 3.7 as the mean ± SEM. There was a significant main effect of STIMULUS INTENSITY (F=33.47; df=1.11, 0.33; p=0.0005). Within-subjects contrasts showed that there was a significant increase in amplitude between the 12 Mcd and 16 Mcd stimulus intensity (F=23.16; df=1, 0.25; p=0.0005), between the 12 Mcd and 20 Mcd stimulus intensity (F=36.48; df=1, 0.35; p=0.0005) and between the 16 Mcd and 20 Mcd stimulus intensity (F=30.50; df=1; p=0.0005). There was no significant main effect of STRAIN (F=0.12; df=2, 0.0005; p=0.988). There was no significant STIMULUS INTENSITY x STRAIN interaction (F=1.40; df=2.23, 0.40; p=0.252).

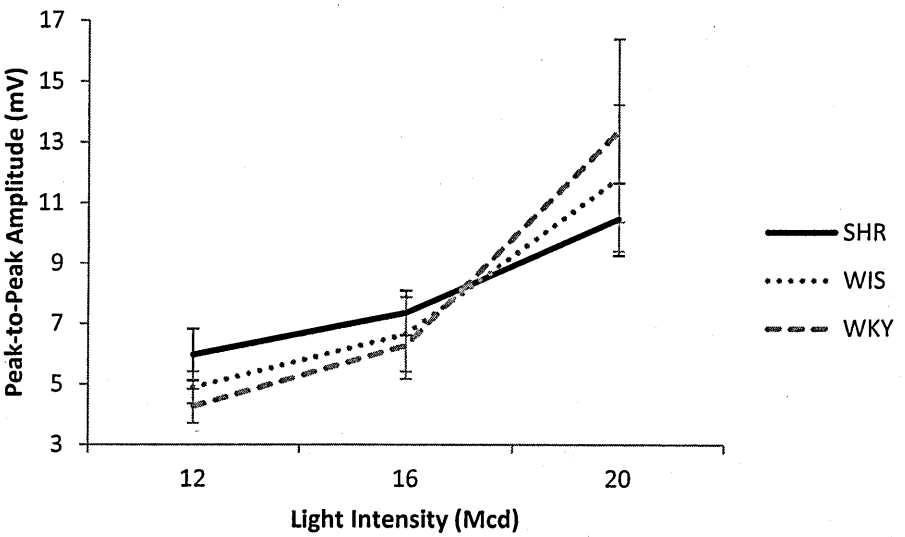

Figure 3.7: The mean ± SEM LFP visual response peak-to-peak amplitude of the three strains over the increasing stimulus intensities showing a significant main effect of intensity with an increase in amplitude as the intensity increased. There were no significant main effects of strain or interactions between strain and light intensity.

Duration

The duration of responses to the stimulus intensities of 12-20 Mcd is shown in Figure 3.8 as the mean ± SEM. Unlike the other parameters, there were no significant main effect of STIMULUS INTENSITY (F=0.81; df=2, 0.12; p=0.447), but there was a significant main effect of STRAIN (F=6.07; df=2, 0.21; p=0.004). Post hoc (Tukey HSD) tests revealed that the WKY

had a significantly longer response duration across all three light intensities in comparison to the SHR (p=0.0.13) and WIS (p=0.007). There was also a trend towards a significant STIMULUS INTENSITY x STRAIN interaction (F=2.52; df=3.47, 0.07; p=0.053). Restricted ANOVAs indicate that this trend is possibly to be due to a lack of strain differences at the greatest stimulus intensity, likely as a result of the greater variation found in the duration of the WKY response at this intensity, as repeated measures ANOVA of all three strains between 12 Mcd and 16 Mcd showed no significant STIMULUS INTENSITY x STRAIN interaction (F=1.00; df=2; p=0.372). Similarly, a one way ANOVA at the 20 Mcd found no main effect of STRAIN (F=0.01; df=1; p=0.909).

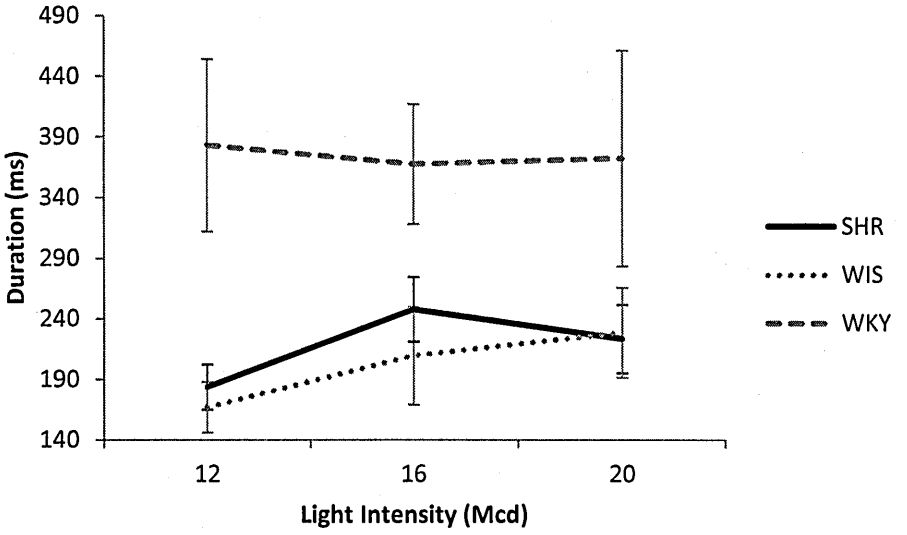

Figure 3.8: The mean ± SEM duration of the LFP visual response of the three strains over the increasing stimulus intensities. No main effect of intensity on response duration was seen. The WKY did have significantly longer response duration than the SHR and WIS. There was a trend towards a significant STIMULUS INTENSITY x STRAIN interaction, this trend is likely to be due to a lack of strain differences at the greatest stimulus intensity, possibly as a result of the greater variation found in the duration of the WKY response at this intensity.

In summary, local field potential responses to visual stimuli revealed no significant differences between the SHR and control stains in terms of the level of responsiveness to the different stimulus intensities measured by onset latency and amplitude. The SHR did differ in response duration, having a decreased duration, but this difference was only found with reference to one of the control strains.

3.3.2.2. VISUAL STIMULATION MULTIUNIT RECORDINGS

Stimulus – response relationship

As with the local field potential recordings, multiunit responses were recorded to five different intensities of a visual stimulus as outlined in Section 2.3.4 and an example of a multiunit response at the middle intensity of a stimulus-response curve for each strain is shown in Figure 3.9 A response was deemed to have occurred if the level of activity rose above a threshold +1.96 SD above the mean, for at least 5 ms (5 consecutive bins). Based on these criteria not all animals responded to all stimulus intensities.

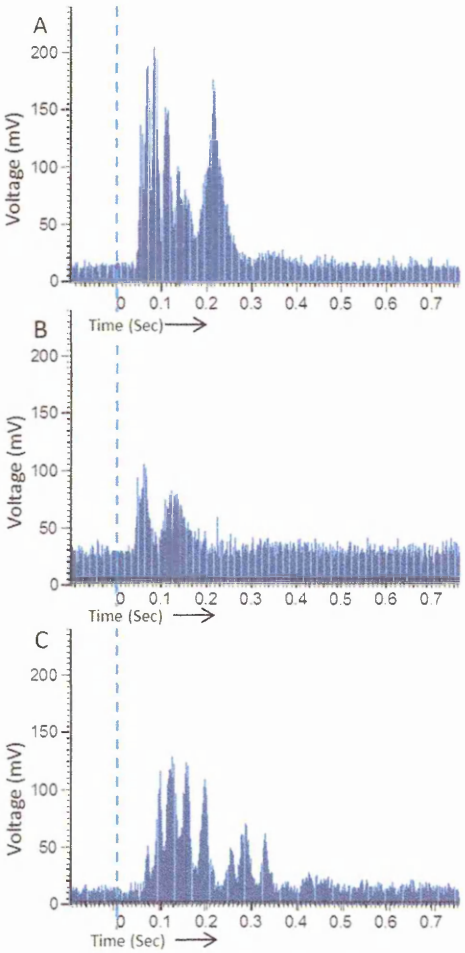

Figure 3.9: An example of a visual response multiunit activity PSTH at the middle intensity of a stimulus-response curve: A: SHR; B: WIS; C: WKY. The dotted blue line represents the point in time when the stimulus occurred.

The percentage of animals responding at each intensity is shown by strain in Table 3.5 and Figure 3.10, where stimulus intensity 4 Mcd is the lowest intensity and stimulus intensity

20 Mcd is the highest. Animals were least likely to respond at the lowest and second lowest intensities where maximum responsiveness was 70.0 % and 75.9 % respectively. A Chi-Square analysis revealed a significant association between strain and likelihood of responding at the intensities 4 Mcd- 12 Mcd where the SHR was most likely to respond (see Table 3.5 for statistics). By 16 Mcd intensity the majority of animals were responding and there were no differences in likelihood of responses between the strains.

Stimulus intensity (Mcd)	Percentage of animals responding			Analysis of strain differences		
	SHR(n=27)	WIS (n=30)	WKY(n=29)	χ^2	df	p
4	70.0	30.0	37.9	10.32	2	0.006
8	75.9	43.3	51.7	9.24	2	0.010
12	100	73.3	89.7	9.29	2	0.010
16	100	93.3	96.6	1.88	2	0.391
20	100	100	100	N/A		

Table 3.5: The percentage of rats that responded at each light intensity. There was a significant difference in strain at the first three intensities, where the SHR was more likely to respond.

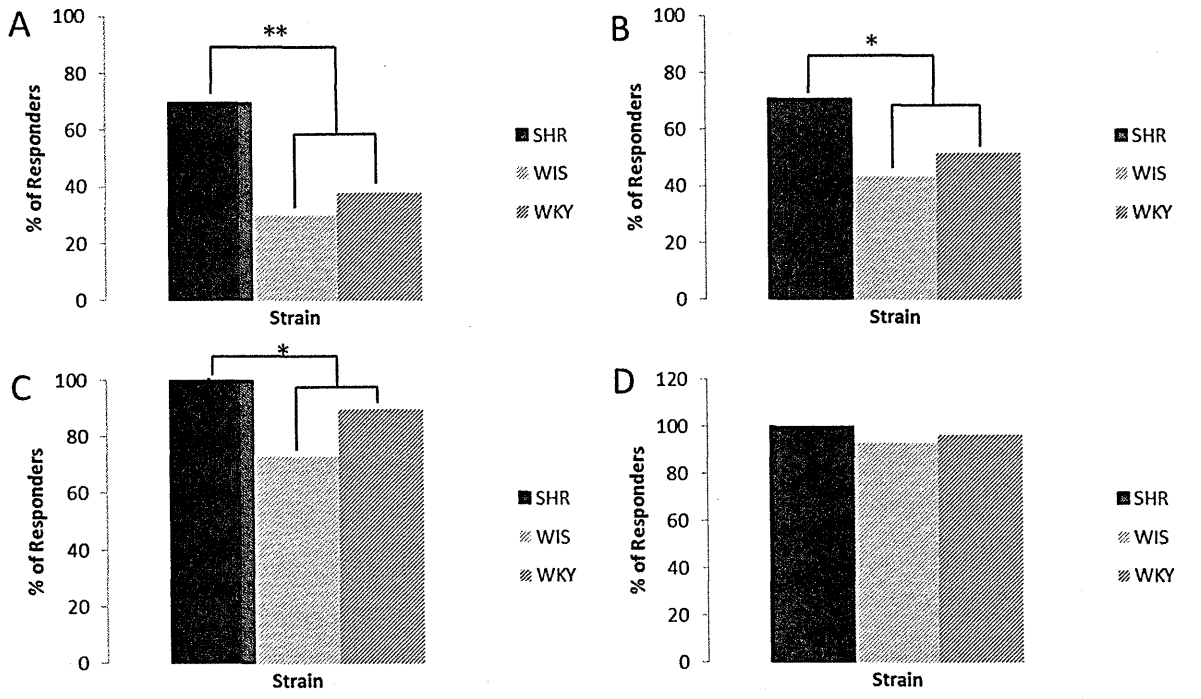

Figure 3.10: The percentage of animals that produced a multiunit activity light response to the lowest four light levels (by the highest level all responded); A: 4 Mcd; B: 8 Mcd; C: 12 Mcd; D: 16 Mcd. The SHR were significantly more likely to respond than the two control strains at stimulus light intensities of 4-12 Mcd (* $p<0.05$; ** $p<0.005$).

In order to look at effects of stimulus intensity, data from only the final three intensities were analysed. For the 12 Mcd light intensity, 8 WIS and 3 WKYs did not respond, so were excluded from the analysis (parameters: onset latency; peak-to-peak amplitude; duration; final N: SHR n=27; WIS n=22; WKY n=26). By the 16 Mcd stimulus intensity all animals responded other than 2 WIS and 1 WKY. All animals responded to the 20 Mcd stimulus.

Onset latency

The onset latency for responses to the stimulus intensities of 12-20 Mcd is shown in Figure 3.11 as the mean ± SEM. There was a significant main effect of STIMULUS INTENSITY (F=59.79; df=1.43, 0.45; p=0.0005). Within-subjects contrasts showed that there was a significant decrease in onset latency between the 12 Mcd and 16 Mcd stimulus intensity (F=20.90; df=1, 0.23; p=0.0005), 12 Mcd and 20 Mcd intensity (F=90.04; df=1, 0.56; p=0.0005) and 16 Mcd and 20 Mcd intensity (F=37.81; df=1; p=0.0005). There was a significant main effect of STRAIN (F=4.06; df=2, 0.10; p=0.021). Post hoc (Tukey HSD) tests showed that the SHR had a significantly greater onset latency in comparison to the WKY (p=0.022) but not the WIS (p=0.114). There was no significant STIMULUS INTENSITY x STRAIN interaction (F=0.56; df=2.85, 0.02; p=0.637).

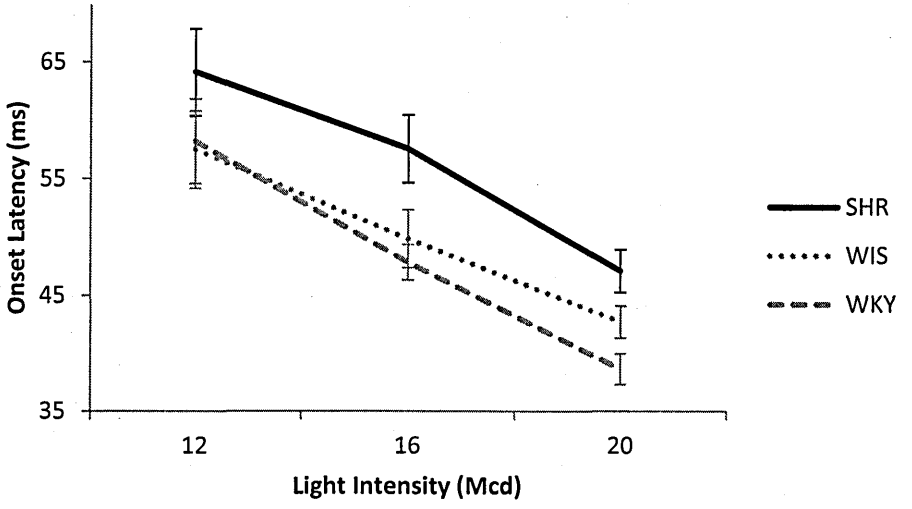

Figure 3.11: The mean ± SEM multiunit activity visual response onset latency of the three strains over the increasing stimulus intensity showing a decline in onset latency as the intensity increased. The SHR had significantly greater onset latencies than the WKY but not the WIS. There were no interactions between strain and intensity.

Amplitude

The amplitude for responses to the stimulus intensities of 12-20 Mcd is shown in Figure 3.12 as the mean ± SEM. There was a significant main effect of STIMULUS INTENSITY (F=135.56; df=1.54, 0.65; p=0.0005). Within-subjects contrasts showed that there was a significant increase in amplitude between the 12 Mcd and 16 Mcd stimulus intensity (F=72.33; df=1, 0.50; p=0.0005), 12 Mcd and 20 Mcd intensity (F=176.60; df=1, 0.71; p=0.0005), and 16 Mcd and 20 Mcd intensity (F=22.47; df=1; p=0.0005). There was no significant main effect of STRAIN (F=1.15; df=2, 0.03; p=0.32). There was a significant STIMULUS INTENSITY x STRAIN interaction (F=4.47; df=3.08, 0.11; p=0.005) for this parameter. Restricted ANOVAs indicate that this interaction was due to the SHR having a lesser increase in amplitude at increasing intensities in comparison to the control strains. One way ANOVAs revealed at the 12 Mcd intensity, the SHR had a trend towards a significantly greater amplitude in comparison to the WKY (F=3.83; df=1; p=0.056), and similarly at the 16 Mcd intensity with the WIS (F=3.89; df=1; p=0.055). By the final intensity (20 Mcd), the SHR did not differ in comparison to either control strain. Repeated measures ANOVAs also revealed that there was a significant STIMULUS INTENSITY x STRAIN interaction between the SHR and WIS between the 16 Mcd and 20 Mcd intensity (F=9.69; df=1, 0.17; p=0.003), as well as the SHR and WKY at the 16 Mcd and 20 Mcd intensities (F=69.17; df=1, 0.58; p=0.0005), where the two control strains showed a greater increase in response than the SHR between the 16 Mcd and 20 Mcd intensity. No strain differences by the final intensity could suggest a plateauing effect, and maximal response had been reached.

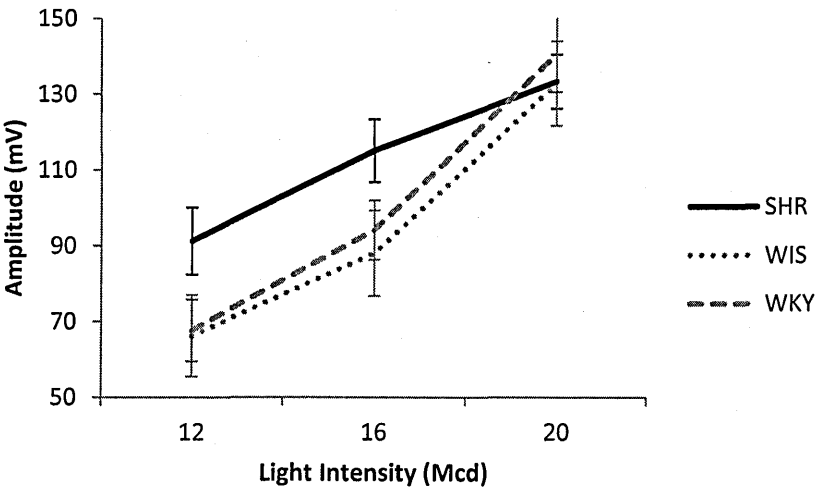

Figure 3.12: The mean ± SEM multiunit activity visual response amplitude of the three strains over the increasing stimulus intensity showing an increase in amplitude as the intensity increased. There were no strain differences, but there was a significant difference in interactions of strain and intensity, with the SHR having larger amplitude responses only at lower intensities.

Duration

The duration for responses to the stimulus intensities of 12-20 Mcd is shown in Figure 3.13 as the mean ± SEM. There was a significant main effect of STIMULUS INTENSITY (F=38.01; df=1.59, 0.35; p=0.0005). Within-measures subjects showed that there was a significant increase in duration between the 12 Mcd and 16 Mcd stimulus intensity (F=40.43; df=1, 0.36; p=0.0005), the 12 Mcd and 20 Mcd stimulus (F=52.62; df=1, 0.42; p=0.0005) and the 16 Mcd and 20 Mcd intensity (F=15.54; df=1; p=0.0005). There was a main effect of STRAIN (F=5.27; df=2, 0.13; p=0.007). Post hoc (Tukey HSD) tests showed the WKY had a significantly longer response duration than WIS (p=0.005), but this was not significant compared to the SHR. There was no significant STIMULUS INTENSITY x STRAIN interaction (F=0.44; df=3.18, 0.12; p=0.740).

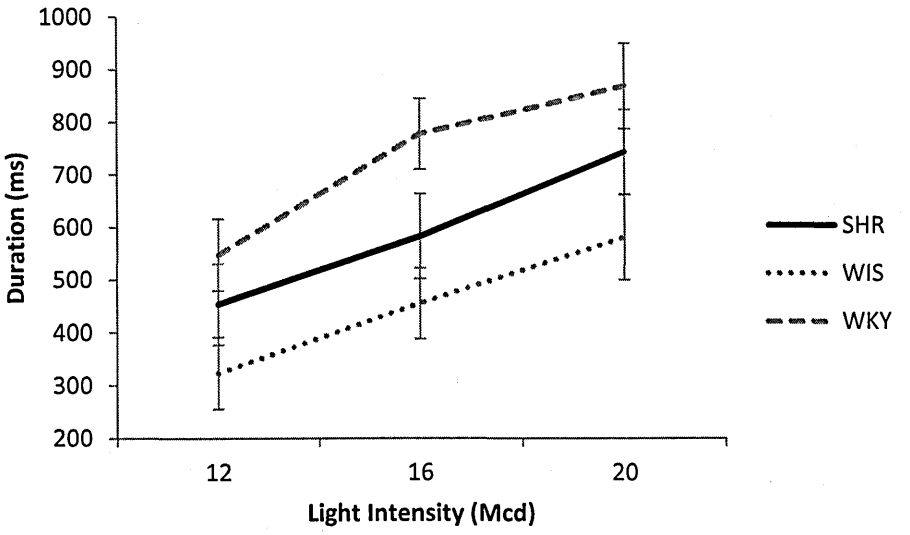

Figure 3.13: The mean ± SEM multiunit activity visual response duration of the three strains over the increasing stimulus intensity, showing an increase in response duration as the intensity increased. The WKY had significantly longer response duration than the WIS, yet not the SHR. There was no interaction between strain and intensity.

In summary, multiunit activity responses to visual stimuli revealed that the SHR was significantly more likely to respond than the control strains at the first three intensities. There were no significant differences between the SHR and control stains in terms of response duration. The SHR did differ in response onset latency, having a increased onset latency, but this difference was only found with reference to one of the control strains. There was a significant interaction in amplitude between the SHR and the two control strains between the 16 Mcd and 20 Mcd intensity, with SHRs showing larger amplitude responses at lower intensities. There were no significant differences between the SHR and control strains by the final intensity and this could suggest a plateauing effect, and maximal response had been reached.

3.3.3. VOLUME ESTIMATES AND FRACTION

Volume estimates

The volume of the superficial layers of the colliculus was estimated using the Cavalieri method as described in Chapter 2 (Section 2.4.2). The SHR had the smallest estimated volume (0.92±0.03 x10^{10} μm^3) in comparison to the WIS (1.10±0.04 x10^{10} μm^3) and WKY

(1.16±0.04 x10^{10} µm^3) as shown in Figure 3.14a. A strain comparison made using a One-Way ANOVA revealed a significant difference between the three strains (F=10.70; df=2; p=0.001). Post hoc (Tukey HSD) tests showed that the SHR had a significantly smaller superficial layer collicular volume than the WIS (p=0.011) and WKY (p=0.001). There was no significant difference between the WIS and WKY (p=0.559). However, despite no strain differences in overall body weight for this experiment, analysis of whole brain volume using a One-Way ANOVA also revealed a significant difference between the three strains (F=3.86; df=2; p=0.044; Figure 3.14b). Post hoc (Tukey HSD) tests revealed that the SHR had a significantly smaller whole brain volume than the WIS (p=0.041) but not the WKY (p=0.172). There was no significant difference between the WIS and WKY (p=0.707). Given these differences in whole brain volume, it was necessary to normalise collicular volume to whole brain volume, and therefore calculate a volume fraction for a more reliable analysis.

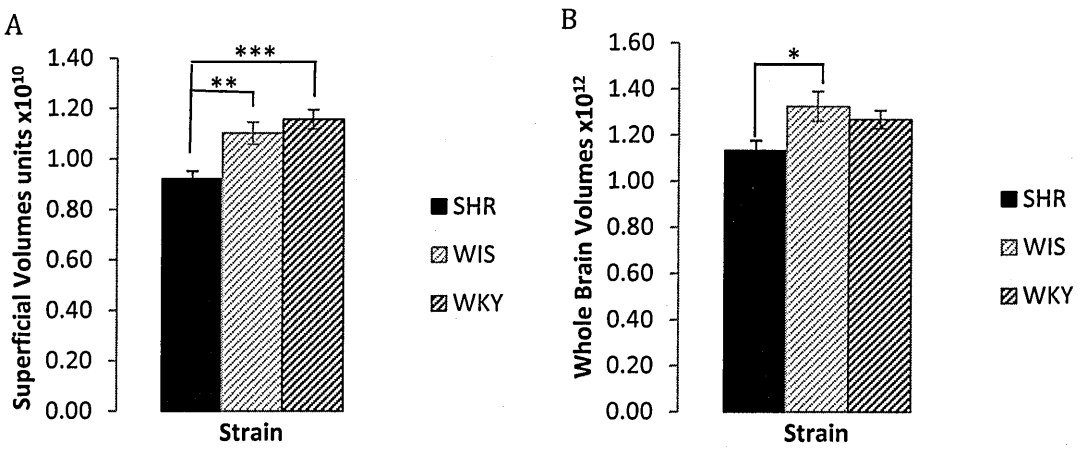

Figure 3.14: The mean ± SEM estimated volumes of the areas for the three strains. A: Superficial layer volume estimates. B: Whole brain volume estimates. The SHR had significantly smaller brains than the WIS, and significantly smaller superficial SC layers than both the WKY and WIS. (* p<0.05; ** p<0.005; p<0.0005).

Volume fraction

When the volume fraction was compared between strains using a One-Way ANOVA there was no significant difference (F=1.16; df=2; p=0.341). The superficial layers of the SC were 0.77±0.04% of the SHR total brain volume; similarly to the WIS and WKY with a volume fraction of 0.79±0.02% and 0.84±0.03% respectively (Figure 3.15).

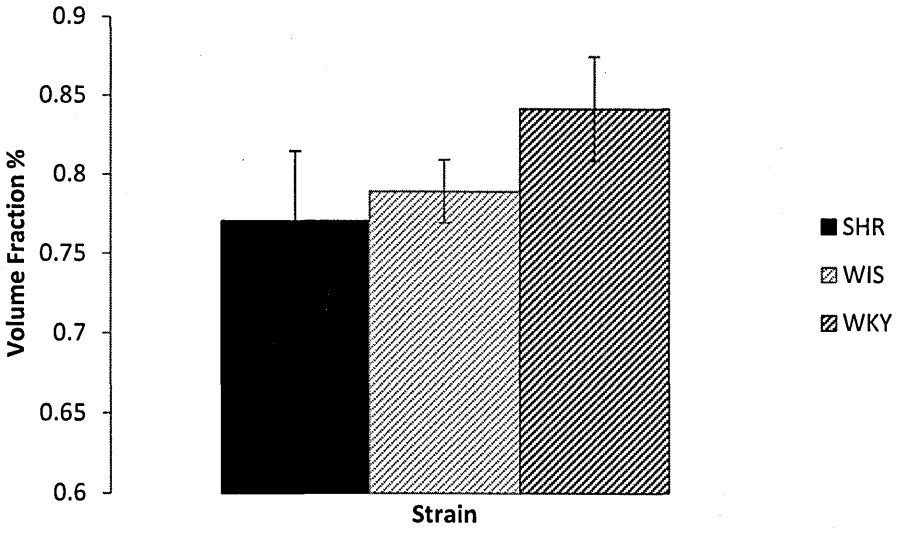

Figure 3.15: The volume fraction of the superficial layer volumes in reference to the whole brain volumes. No significant differences were found.

3.3.4. CELL COUNTS AND DENSITY

Cell counts

Cell counts in the superficial layers were measured as described in Chapter 2 (Section 2.4.3) and are shown in Figure 3.16. A strain comparison made using a One-Way ANOVA revealed no significant difference between the three strains in the total number of cells ($F=2.82$; $df=2$; $p=0.094$) or amount of neurons ($F=0.58$; $df=2$; $p=0.572$). However, there was a significant difference in the amount of glia ($F=4.23$; $df=2$; $p=0.037$). Post hoc (Tukey HSD) tests revealed that the SHR had significantly less glia than the WKY ($p=0.038$) but not the WIS ($p=0.126$) and there was no significant difference between the WIS and WKY ($p=0.853$).

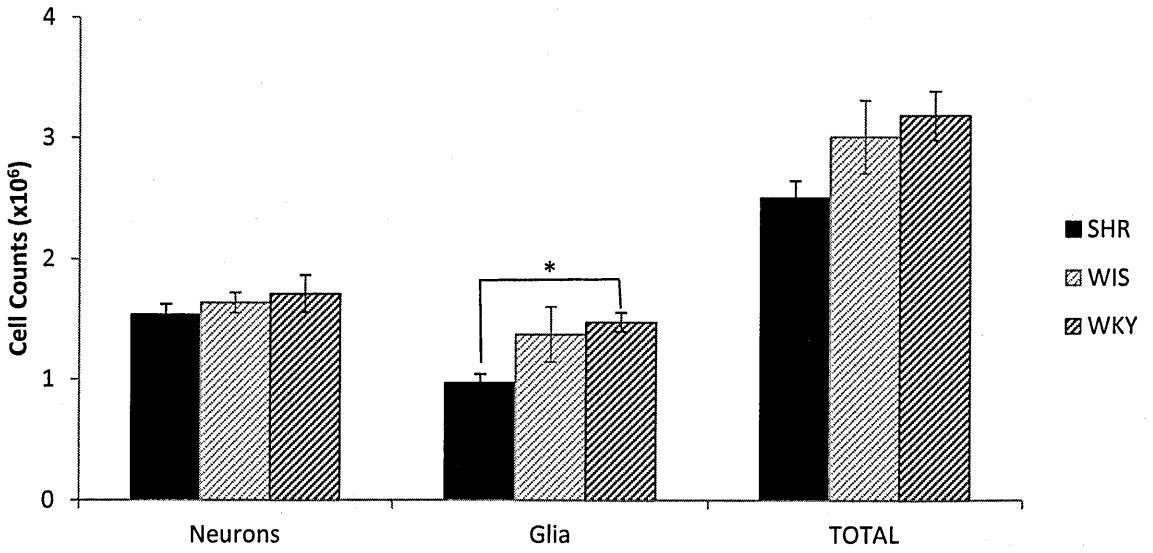

Figure 3.16: The mean ± SEM estimated cell counts, of neurons, glia and total cell counts within the superficial layers of the SC of the three strains. The SHR had significantly less glia than the WKY. (* $p<0.05$).

Neuron: glia ratio

Neuron: glia ratio is shown in Figure 3.17. A strain comparison made using a One-Way ANOVA, revealed a significant difference in this ratio ($F=4.37$; $df=2$; $p=0.032$). Post hoc (Tukey HSD) tests revealed that the SHR had significantly greater ratio than the WKY ($p=0.030$) but not the WIS ($p=0.169$) and there was no significant difference between the WIS and WKY ($p=0.721$). These findings are likely due to the significantly greater numbers of glia cells in the SHR compared to the WKY.

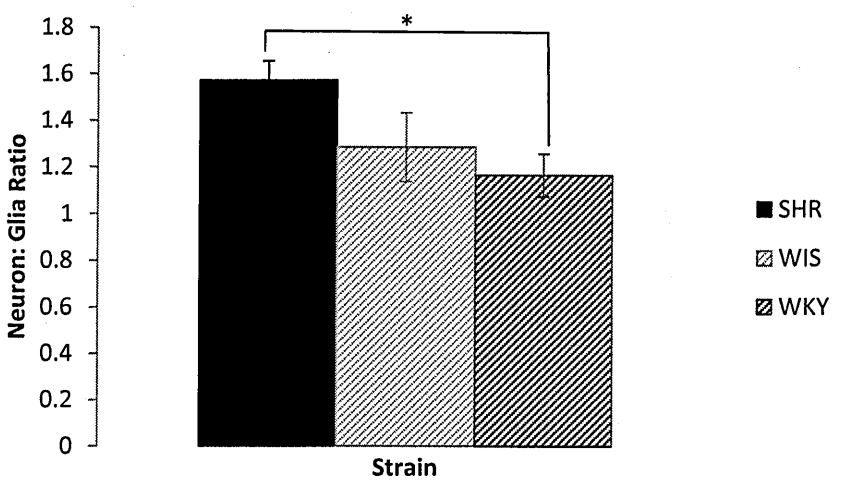

Figure 3.17: Neurons: glia ratio in the superficial layers. The SHR had a significantly greater ratio than the WKY, but not the WIS. (* $p<0.05$)

Cell density

Cell densities in the superficial layers were measured as described in Chapter 2 (Section 2.4.3) and are shown in Figure 3.18. A strain comparison made using a One-Way ANOVA revealed no significant difference between the three strains in the density of neurons ($F=1.40$; $df=2$; $p=0.279$) or glia ($F=0.56$; $df=2$; $p=0.582$) or total cell densities ($F=0.043$; $df=2$; $p=0.958$).

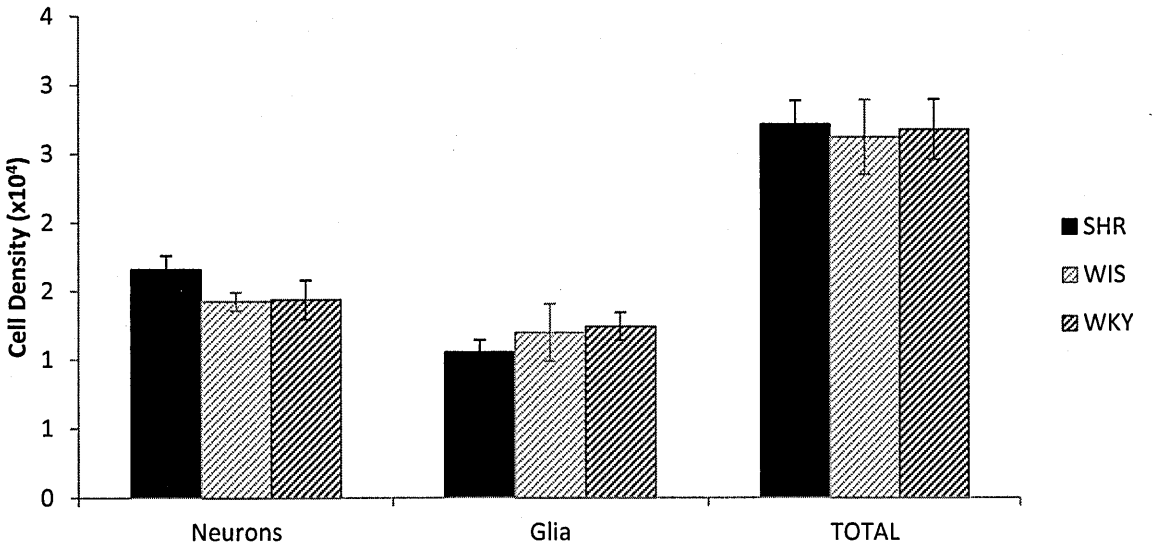

Figure 3.18: The mean ± SEM cell density of neurons, glia and all cells in the superficial layers. There were no significant differences found.

In summary, the morphological analysis of the superficial layers of the colliculus revealed that the SHR had significantly smaller brain volumes but this difference was only found with reference to one of the control strains. The SHR had significantly smaller superficial SC layers than both control strains, but the SHR did not differ from the two control strains in the volume fraction of the SC superficial layers for all three strains. The SHR had significantly less glia in comparison to one control strain, as well as having a greater neuron: glia ratio than one control strain, but the SHR did not differ to the two control strains in cell densities.

3.4. DISCUSSION

In the SC-dependent behavioural task, the SHR did not habituate to a visual stimulus in a similar way to the two control strains, instead showing persistent responding to the stimulus and for a greater duration throughout the ten trials. In the electrophysiological study of visual responsiveness, as expected, an increase in light intensity caused an enhancement in both local field potential (decrease in onset latency, increase in amplitude) and multiunit activity (decrease in onset latency, increase in amplitude and duration) responses for all strains. There was no strain difference in the likelihood to respond at any light intensity for the local field potential responses but the SHR was significantly more likely to produce a multiunit activity response to the first three intensities than the WKY or WIS. The WKY had a significantly longer LFP response duration than the WIS and SHR. Similarly the WKY had significantly longer multiunit activity response duration than the WIS but not the SHR. There was a significant interaction in multiunit activity amplitude between the SHR and the WKY or WIS, with the SHR showing larger responses at lower intensities. No strain differences by the final intensity could suggest a plateauing effect, and maximal response had been reached. The structural analysis of the superficial layers of the colliculus revealed that the SHR had significantly smaller brains than the WIS and significantly smaller superficial SC layers than both control strains, yet there were no significant differences in the volume fraction of the SC superficial layers for all three strains. The SHR had significantly less glia in comparison to the WKY only, as well as having a greater neuron: glia ratio than the WKY. However, when brain volume was taken into account by examining cell density, there were no significant strain differences.

In the SC-dependent behavioural task the SHR was found to show longer duration responses to each individual stimulus presentation and to have impaired habituation to the repeated presentation of the non-novel, non-salient stimuli. It should be mentioned that in the locomotor activity task (see Section 2.2.2), the SHR showed significantly more vertical

activity than the WKY, yet not the WIS. Similarly the WKY moved significantly less distance than the WIS and SHR. Yet the animals did not have to move towards the stimulus or be within a certain area of the arena to respond to the stimulus, so therefore distance travelled would not confound results. Similarly, vertical movements were not a common occurrence in the classification of a response, so would not confound results. It is also worth noting that the SHR showed behavioural differences in the persistence of responding to the stimulus and for a greater duration throughout the ten trials in comparison to both control strains, and not just the WKY, which is the strain that differed in locomotor activity. This heightened responsiveness, in terms of duration and the lack of habituation in comparison to the two control strains could arise from dysfunction within the SC processing of stimulus saliency (see Section 1.2.5). A heightened SC response would give a 'stronger bid' to the basal ganglia, increasing the chance of responding to the stimuli, and thus the behaviours seen in the behavioural task. As previously mentioned, SC-lesioned rats are deficient in problem solving when the correct functioning of orienting behaviour and attentional processing is needed (Weldon and Smith, 1979), emphasising the importance of the SC interaction with other neural systems in processes mediating the direction of attentional focus. An increase in the perception of the saliency of stimuli could also be an underlying cause of impairments in behavioural inhibition. Similarly, dysfunction of behavioural inhibition (see Section 1.3.2.1) as well as intolerance to delay of reinforcement (see Section 1.3.2.1) has been shown behaviourally in the SHR. This lack of behavioural inhibition could suggest the findings in the present behavioural task, where the SHR were simply unable to inhibit unnecessary behavioural responses in order to habituate to stimuli that is neither salient or novel, both having strong links to the functional role of the SC. The behavioural results suggest that the SC might be hyper-responsive, yet whether this is due to a hyper-responsive SC or due to increased inputs from areas upstream is unclear.

Also in the present study, the SHR was significantly more likely to show a multiunit activity response to the lower three light levels than the two control strains and larger multiunit responses at lower intensities. As no strain differences were found at the final intensity, this suggests a maximal response occurring, and thus a plateauing effect for all strains. As there was no significant increase in the LFP visual response (input) in the SHR compared to the controls, it suggests that there is a difference in the processing of the visual information within the SC, producing a significant increase in the multiunit activity response (output) in the SHR. The finding that the SHR are physiologically more responsive to light in this way could be a reason for the increased distraction and lack of behavioural habituation to non-novel, non-salient stimuli seen in the SC-dependent behavioural task mentioned above. It has been hypothesised that the SC could 'bid' for motor expression, thus, heightened activity can be thought as placing a stronger "bid' into the central selection device thought to be the basal ganglia (Chevalier and Deniau, 1990), therefore increasing the likelihood of saccade generation and orientation towards a stimulus. As the hyper-response was only seen at the weak intensities, yet not seen at the higher intensities could suggest an issue in the signal-to-noise ratio outlined in Dommett et al. (2009), yet biasing the system in an opposing way by enhancing weak and preserving strong activations. The behavioral effects of this could be linked to a change in the signal-to-noise ratio effect mediated in the SC by biasing the system towards non salient stimuli and consequently leading to an increase in distractibility, and could underlie key processes involved in the adaption of the reactivity according to the state of arousal of the animal. A similar effect could be occurring in people with ADHD, as the SC is conserved across species and would therefore cause an increase in overall distractibility and a deficit in sustained attention.

In the present study, the SHR had greater multiunit activity onset latency response to light in the superficial layers in comparison to the WIS, as this occurred only for the multiunit activity response (outputs) and not the LFP response (inputs), it suggests that this finding is

due to a lack of development within the SC rather than upstream of it in these animals. Interestingly, a study in the development of synaptic transmission in the retinocollicluar pathway of the rat found a quickening of onset latencies as this system developed (Reece and Lim, 1988). This finding could suggest that the SHR has a development delay of this system, and may lead to the greater onset latencies seen in these animals. A quickening of onset latency is similarly found in the visual system as it develops in humans (Crognale et al., 2001). Significantly longer saccade latencies and duration in visually guided saccades (VGS, Mahone et al., 2009; Goto et al., 2010), memory guided saccades (MGS, Goto et al., 2010), prosaccades (Klein et al., 2003; Munoz et al., 2003), and antisaccades (Munoz et al., 2003; Feifel et al., 2004; Karatekin, 2006; Karatekin et al., 2010) has been shown in children with ADHD in oculomotor paradigms, a model which is highly dependent on the SC. ADHD is a developmental disorder, with childhood onset (see Section 1.1.1 and Section 1.1.4), therefore a developmental delay of the SC leading to deficient SC processing could link the longer onset latencies in the SC of the SHR and the saccade latencies seen in children with ADHD as well as the behavioural deficits seen in the SHR and children with ADHD.

As previously mentioned in Section 1.2.5, the SC plays a crucial role in the orientation of the head to novel stimuli. In many instances retina and visual cortex afferents synapse on the same neurons in the superficial layers of the SC allowing virtually raw visual retinal information to converge with processed information from the lateral geniculate nucleus (LGN) and visual cortex. In turn, the deeper layers of the SC receive the majority of its visual information from the superficial layers. As Helms et al. (2004) demonstrated, superficial layer neuronal stimulation evokes individual excitatory postsynaptic currents in intermediate layer premotor SC cells, suggesting an influential, monosynaptic, excitatory pathway connecting these layers; which have been shown to be glutamatergic (Isa et al., 1998).

As previously mentioned in Section 1.1.6, the underlying theories of ADHD focus on behavioural inhibition, and dysregulation of dopamine. The electrophysiological findings in the current study suggest that the dysregulation of dopamine may be a secondary effect of a dysfunction in the initial processing of salient stimuli within the SC affecting target selection based on saliency (Kundsen, 2011; Shen et al., 2011), causing impairments in behavioural inhibition to non-salient stimuli in these animals, and potentially in individuals with ADHD. The visual SC response latencies seen in this chapter are consistent with findings from other researchers (40–60 ms, Gowan et al., 2008; Wurtz and Albano, 1980; Munoz and Guitton, 1986; Peck, 1990; Stein and Meredith, 1993). Short latency visual evoked potentials recorded locally in the substantia nigra pars compacta arise from the SC (Comoli et al., 2003), with the SC response latencies are shorter those of dopaminerguc neurons (70–100 ms, Schultz, 1998; Morris et al., 2004; Takikawa et al., 2004). Bilateral removal of the SC caused rats to show no orienting reflex or distraction to the presentation of novel visual or auditory stimuli (Goodale and Murison, 1975). However, visual-decorticated rats did maintain the orienting reflex to novel stimuli (Goodale and Murison, 1975). With detection and object recognition cortical response latencies being longer (80–100 ms) than the response latencies of DA neurons (Thorpe and Fabre-Thorpe, 2001), it suggests that the SC mediates the shift in visual fixation and attention mediated via a direct tectonigral projection (Comoli et al., 2003), while the visual cortex contributes to visuospatial guidance of locomotor movements, but does not play a significant role in the control and integration of the orienting reflex (Goodale and Murison, 1975). Similarly, the SC has the capacity to over-ride and manipulate top-down influences from fronto-parietal network (Wardak et al., 2004, 2006; Lovejoy and Krauzlis, 2010). The increased sensitivity of multiunit activity response to light in the superficial layers of the SC seen the SHR, will cause an increased saliency attributed to stimuli. The SC capacity to activate and modulate dopaminergic phasic activity (Dommett et al., 2005; Coizet et al., 2006) will mean the increase in SC response seen in the SHR will produce secondary effects in the phasic release

of dopamine. This will lead to an increase in the perception of the saliency of stimuli, and cause the lack of habituation to non-salient stimuli seen in these animals in the behavioural task. As there was no significant increase in the LFP visual response (input) in the SHR compared to the controls, it suggests that there is a change in the processing of the visual information within the SC, and potentially with the signal-to-noise ratio in the SC (Dommett et al., 2009) producing a significant increase in the multiunit activity response (output) in the SHR. The capacity to over-ride now invalid prepotent automatic behaviours in accordance to novel situational demand is vital for all mammals (Fernandez-Dugue et al., 2000), as the SC is highly conserved across species, the findings of this chapter can arguably propose similar dysfunctions in individuals with ADHD.

In the present morphological study, the SHR was found to have significantly smaller total brain volume and thus, smaller superior colliculi than the two control strains. It is worth noting that these volume and cell count studies morphological study potentially have low statistical power due to the low number of subjects used. A study with low statistical power has a reduced chance of detecting a true significant effect, and also reduces the likelihood that a statistically significant result reflects a true effect (Button et al., 2013). However, the results of this volume study correspond to similar findings by Nelson et al. (1993), who also found the SHR to have smaller total brain volumes in comparison to the WKY. It also supports the validity of the SHR as an animal model of ADHD because similar reductions in whole brain volume have been shown in the brains of individuals with ADHD, yet no difference in body weight (Castellanos et al., 2002). It is worth noting here that there was no difference in body weight of the strains in the morphological study, so a reduction in brain volume was not due to a reduction in general body size. In the present study, the SHR were also found to have significantly less glia, and therefore a greater neuron: glia cell ratio. However, due to the significant differences in brain volume seen in these animals, cell densities provide a better measure. Also, as no significant differences were found in cell

densities, it must be assumed that the response differences found in the superficial layers of the SC in the SHR must be due to either differences in receptor densities, or differences in the number of different types of neuronal cells (i.e. a greater number of excitatory inputs compared to inhibitory ones, rather than simply the number of neurons or glia).

4. COLLICULAR AUDITORY RESPONSES

This chapter describes the findings from an investigation comparing the functioning and structure of the deeper layers (intermediate and deep) of the SC in the SHR model of ADHD and two control strains. Previously, it was suggested that abnormalities in the SC could result in the symptoms of ADHD and that it should be possible to investigate these abnormalities in the SHR. Therefore, in the present study, it was hypothesised there will be behavioural differences in how the SHR responds and habituates to a visual stimulus in an SC-dependent task. In order to ensure the task was dependent on the intermediate layers, this was tested using an auditory distractor task (Section 2.2.1). Secondly, it was hypothesised that there would be significant differences in the physiological responses to auditory stimuli between the SHR and control strains. As with the behavioural testing, this was investigated by recording responses to auditory stimuli from the intermediate layers of the SC in the anaesthetised rat (Section 2.3.4). Finally, it was hypothesised that there would be significant differences in morphological parameters of the SC (neuronal and glia cell counts and density) between the SHR and control strains (Section 2.4.3). The results of these investigations show that behaviourally all strains responded to the stimulus in a similar manner for all 10 trials. Physiologically, the SHR was hypo-responsive to the auditory stimuli with differences in LFP (input) responses only. This indicates that these strain differences arise from changes upstream of the SC, for example in the inferior colliculus. Arguably, because no differences were found in multiunit (output) response, it can be suggested that the SC is still hyper-responsive (similar to the finding of the visual response data) in the SHR, because the SC compensates for the reduced auditory input, causing no significant differences in response outputs or behaviour: responses and behaviours are normalised. In the present study, the SHR was found to have significantly greater onset latency for both LFP and multiunit activity auditory responses. This is also a similar finding within the superficial layer visual responses (Chapter 3) and similarly suggests a delay in development of this system.

4.1. INTRODUCTION

As previously mentioned, the inability to inhibit unnecessary behavioural responses or habituate to stimuli that are neither salient nor novel is found in individuals with ADHD (Section 1.1.1; Quay, 1997; Dimoska et al., 2003) and in the SHR (Section 1.3.2.1; Li et al., 2007; Sagvolden et al., 1993). All vertebrate species must have the capacity to select information for processing based on its relevance to behaviour. The circuitry in the SC integrates information from the frontoparietal network with the assessment of the physical salience of stimuli (Fecteau and Munoz, 2006; Dorris et al., 2007; Shen and Pare, 2007; Mysore et al., 2011). In this respect, a dysfunction in the processing of saliency of stimuli in the SC will cause behavioural deficits such as dysfunctions of behavioural inhibition, increased distractibility and impulsivity.

In auditory processing the external cortex of the inferior colliculus (IC) constitutes one of, if not the most important source of, auditory information to the SC, and appears to contribute considerably to acoustico-motor pathways involved in SC-mediated orienting behaviour. Stimulation of the IC activates auditory superior collicular neurons, eliciting simultaneous movement of the ear in conjunction with contralaterally directed eye movements (Syka and Straschill, 1970; Huffman and Henson, 1990). Electrical or chemical stimulation of the IC induces fear-like reactions such as freezing, fight, or wild running (Cardoso et al., 1994; Pandossio and Brandão, 1999). SC-lesioned rats are also deficient in problem solving when the correct functioning of orienting behaviour and attentional processing towards light stimuli was needed (Weldon and Smith, 1979; Midgley and Tees, 1986). Despite this research looking at a different sensory modality, this emphasises the importance of the SC interaction with other neural systems in processes mediating the direction of attentional focus, as similar roles and connections are found in this multi-sensory structure. The deep layers of the SC receive auditory information from the auditory cortex originated from the dorsal part of the ipsilateral auditory cortical area, in layer V (Druga and Syka, 1984).

However, auditory inputs arise from a variety of ascending sources (primarily contralateral; Edwards et al., 1979; Druga and Syka, 1984). These ascending inputs are denser than the ascending visual processing pathways in the SC. The excitatory cortico-tectal control is required to ensure that SC activity can be modified and modulated by experience and current needs. Disconnecting the SC from the prefrontal cortex controlling influences leads to an increase in distractibility in humans (Gaymard et al., 2003). A decrease in distractibility has also been observed in SC-lesioned animals in an array of species (cat: Sprague and Meikle, 1965; rat: Goodale et al., 1978; monkey: Milner et al., 1978).

In light of this evidence, it is hypothesised that there will be behavioural differences in how the SHR responds and habituates to an auditory stimulus in an SC-dependent task. It is hypothesised that physiological auditory responses in the SC will be different in the SHR, leading to secondary effects in determining the saliency of stimuli, and the ADHD-like distractibility behaviours seen in this strain. Morphological differences in neuronal cell counts are also hypothesised to be cause of these physiological differences.

Hypotheses

- There will be behavioural differences in how the SHR responds and habituates to an auditory stimulus in an SC-dependent task in comparison to the two control strains (WKY and WIS).
- There will be physiological differences in the responses to auditory stimuli recorded in the deeper layers of the SC in the SHR in comparison to the two control strains (WKY and WIS).
- There will be morphological differences in the deeper layers of the SC in the SHR in comparison to the two control strains (WKY and WIS).

4.2. METHODS

A total of 110 rats were used for the experiments described (35 SHR; 36 WIS; 39 WKY). Following the behavioural experiments, the animals were used for electrophysiological experiments, but the animals used for the morphological experiments were singularly used for this alone. The weight of the animals immediately prior to experimentation is detailed, by strain and experiment, in Table 4.1. The normality of the weight data was confirmed using the Kolmogorov–Smirnov test and a One-Way ANOVA was conducted to examine where there were any strain differences in weight for each type of experiment. This revealed a significant difference in weights between the strains for the behavioural experiment ($F=8.39$; $df=2$; $p=0.002$). Post hoc (Tukey HSD) analysis revealed the WIS had a significantly greater weight than the WKY ($p=0.002$) and the SHR ($p=0.026$). There was no significant difference between the WKY and SHR ($p=0.554$). Similar findings were seen in the electrophysiological experiment ($F=47.28$; $df=2$; $p=0.0005$). Post hoc (Tukey HSD) analysis revealed the WIS had a significantly greater weight than the WKY ($p=0.0005$) and the SHR ($p=0.0005$), but that there was no significant difference between the WKY and SHR ($p=0.781$). There was no significant difference in weight between the strains for the cell count experiment ($F=1.33$; $df=2$; $p=0.311$) and volume experiment ($F=3.14$; $df=2$; $p=0.092$).

Experiment		SHR	WIS	WKY
Behaviour task	Number of subjects	9	8	9
	Mean weight ± SEM(g)	399.97±11.17	448.25±10.08	466.34±14.07
Electrophysiology	Number of subjects	27	28	31
	Mean weight ± SEM (g)	389.29±5.82	499.90±67.96	380.15±49.56
Volume Estimation	Number of subjects	4	4	4
	Mean weight ± SEM (g)	396.78±16.24	484.83±28.71	431.00±28.14
Cell counts	Number of subjects	4	4	4
	Mean weight ± SEM (g)	413.85±16.27	455.55±19.47	410.45±27.84

Table 4.1: The mean ± SEM weights and number of subjects for the experiments within this chapter.

Strain differences and the effect of increasing intensity of the auditory stimulus on collicular responses of specific parameters (onset latency, amplitude, duration) were analysed using repeated measures ANOVA with STIMULUS INTENSITY as the within-subjects factor and STRAIN as the between-subjects factor. All data were confirmed as having a normal distribution using the Kolmogorov–Smirnov test before being analysed.

4.3. RESULTS

4.3.1. BEHAVIOUR

Level of responding and habituation

Responsiveness towards the distractor stimulus was defined in Section 2.2.1 of Chapter 2 as if the animal physically interacted with the stimulus, froze or oriented its head towards the stimulus. Using this definition of responding there was a high level of responding on the first presentation of the stimulus with 89% (8/9) SHR; 88% (7/8) WIS and 100% WKY responding towards the auditory stimulus. Overall, this high level of responding is to be expected because the stimulus is novel. Therefore, we examined how the level of responding changed with each consecutive presentation of the stimulus by plotting the percentage of animals responding to the stimulus as a function of stimulus presentation. Figure 4.1a shows that the percentage of animals responding to the stimulus decreases with increased number of stimulus presentations for all three strains. By the final stimulus presentation, the WKY response rate was highest with 78% (7/9) still responding in comparison to 33% (3/9) SHR and 25% (2/8). A survival analysis life table was used to examine the differences between the three strains in terms of this drop in responsiveness, or put another way the habituation to the stimulus over the consecutive stimulus presentations, where TIME was the 5 second epoch of each trial (10 trials), STATUS was whether the animal responded towards the stimulus (1= habituated, 0= responded), and the FACTOR was STRAIN. The median survival time is the time at which 50% of those who originally started out responding have habituated and no longer respond. The SHR had a

median survival time of 9.58, the WKY and WIS had a median survival time of 10.00 and 9.50 respectively. There was no significant difference in STRAIN (F=0.25; df=2; p=0.882).

In addition to looking at the period for which the stimulus was on, the pre- and post-5 second epoch was also examined to ensure the differences found were not due to general behaviour towards the object in the arena (see Figure 4.1 b and c) There was no decline in the level of engagement with the stimulus in the pre- and post- stimulus epochs to the same extent as when the stimulus was ON indicating that the decline in engagement while the stimulus was ON was not due to the animals' behaviour in the arena but was specifically due to stimulus ON interaction.

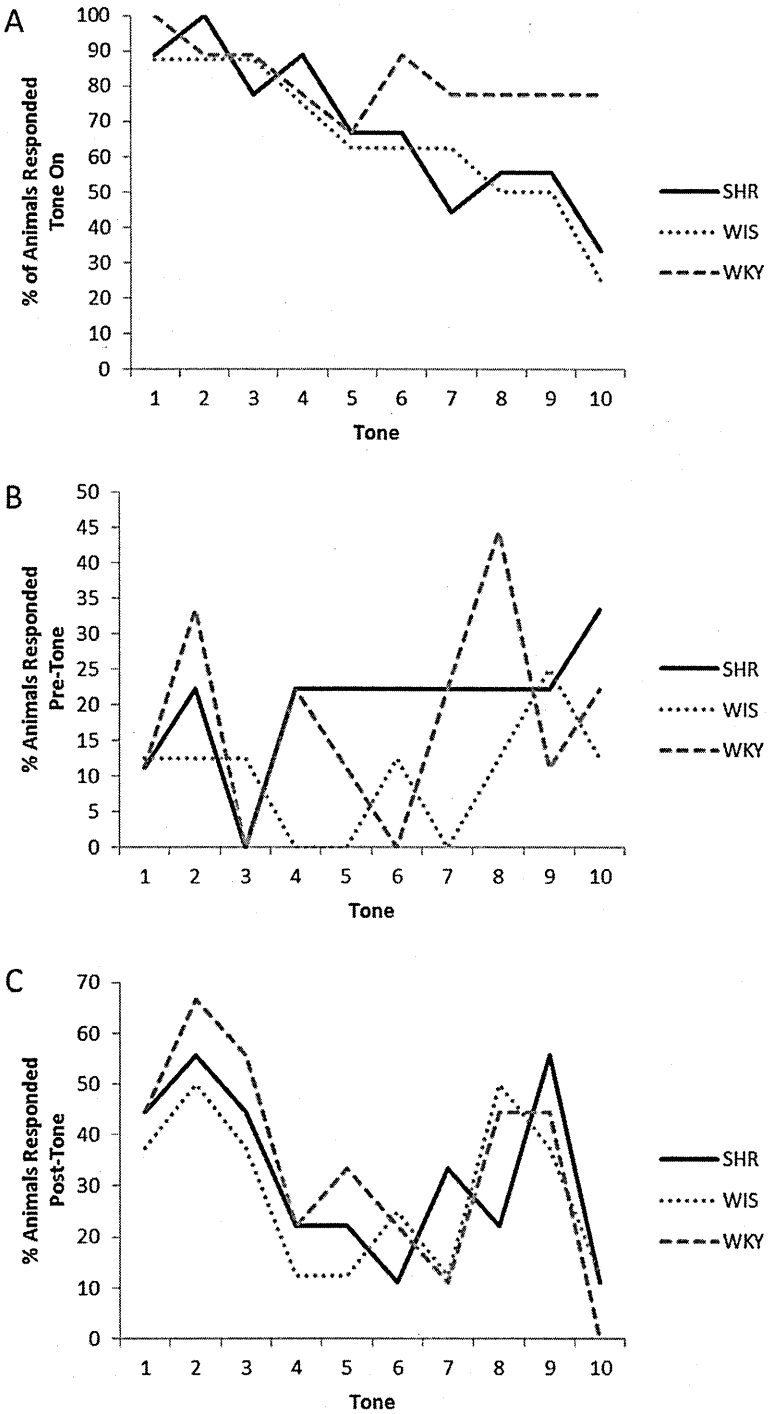

Figure 4.1: All strains responded towards the auditory stimulus or equipment at a similar rate over all 10 trials. A: Pre-stimulus epoch; B: Stimulus ON epoch; C: Post-stimulus epoch.

Duration of response

During the 5 second time period when the auditory stimulus was on, all three strains spent a similar amount of time responding to the stimulus during the first presentation (SHR: 50.67±11.21% of total time or 2.53±0.56 s; WIS: 48.25±10.93% of total time or 2.41±0.58 s;

WKY: 52.00±7.48% of total time or 2.60±0.37 s; see Figure 4.2a). Repeated measures ANOVA with STIMULUS PRESENTATION as the within-subjects factor and STRAIN as the between-subjects factor was conducted using the percentage of overall time responding to the stimulus as the dependent variable. There was a significant main effect of STIMULUS PRESENTATION (F=5.78; df=9, 0.20; p=0.0005; see Figure 4.2a). All animals spent significantly less time with the stimulus as the consecutive trials occurred, with significant time decreases compared to the 1st stimulus beginning at the 6th stimulus (F=13.23; df=1, 0.37; p=0.001). By the final stimulus there was a highly significant time difference in response duration (F=23.34; df=1, 0.50; p=0.0005). There was a trend towards a significant main effect of STRAIN (F=3.27; df=2, 0.22; p=0.056) but post hoc (Tukey HSD) tests showed no significant STRAIN differences (WKY/ WIS: p=0.075; WKY/ SHR: p=0.115; WIS/ SHR p=0.957). There was no significant STIMULUS PRESENTATION x STRAIN interaction (F=0.31; df=18, 0.03; p=0.997).

During the 5 seconds prior to the onset of the auditory stimulus, the animals showed very little interest in the stimulus equipment, spending only brief periods distracted by the equipment (SHR 1.96±1.34% of total time or 0.10±0.07 s; WIS 0.83±0.78% of total time or 0.04±0.04 s; WIS 3.22±2.49% of total time or 0.16±0.12 s) as shown in Figure 4.2b. Repeated measures ANOVA with STIMULUS PRESENTATION as the within-subject factor and STRAIN as the between-subject factor was conducted using the percentage of overall time responding to the stimulus as the dependent variable. There were no significant main effect of STIMULUS PRESENTATION (F=0.74; df=2.12, 0.03; p=0.492; see Figure 4.2b) or STRAIN (F=2.19; df=2, 0.16; p=0.135). There was no significant STIMULUS PRESENTATION x STRAIN interaction (F=0.85; df=4.25, 0.07; p=0.509). The behaviour in the 5 second post-stimulus was also analysed. Similarly to the pre-auditory stimulus time period, all strains spent very little time responding to the stimulus equipment during this time, with the SHR, WIS and WKY spending 4.71±3.28% (0.24±0.16 sec), 4.93±3.49% (0.25±0.17 sec),

8.62±5.14% (0.43±0.26 sec) respectively when averaged across all trials (see Figure 4.2c). Similarly, repeated measures ANOVA with STIMULUS PRESENTATION as the within-subjects factor and STRAIN as the between-subjects factor was conducted using the percentage of overall time responding to the stimulus as the dependent variable. There were no significant main effect of STIMULUS PRESENTATION (F=0.96; df=5.47, 0.04; p=0.449; see Figure 4.2) or STRAIN (F=1.43; df=2, 0.11; p=0.259). There was no significant STIMULUS PRESENTATION x STRAIN interaction (F=0.77; df=10.94, 0.06; p=0.671).

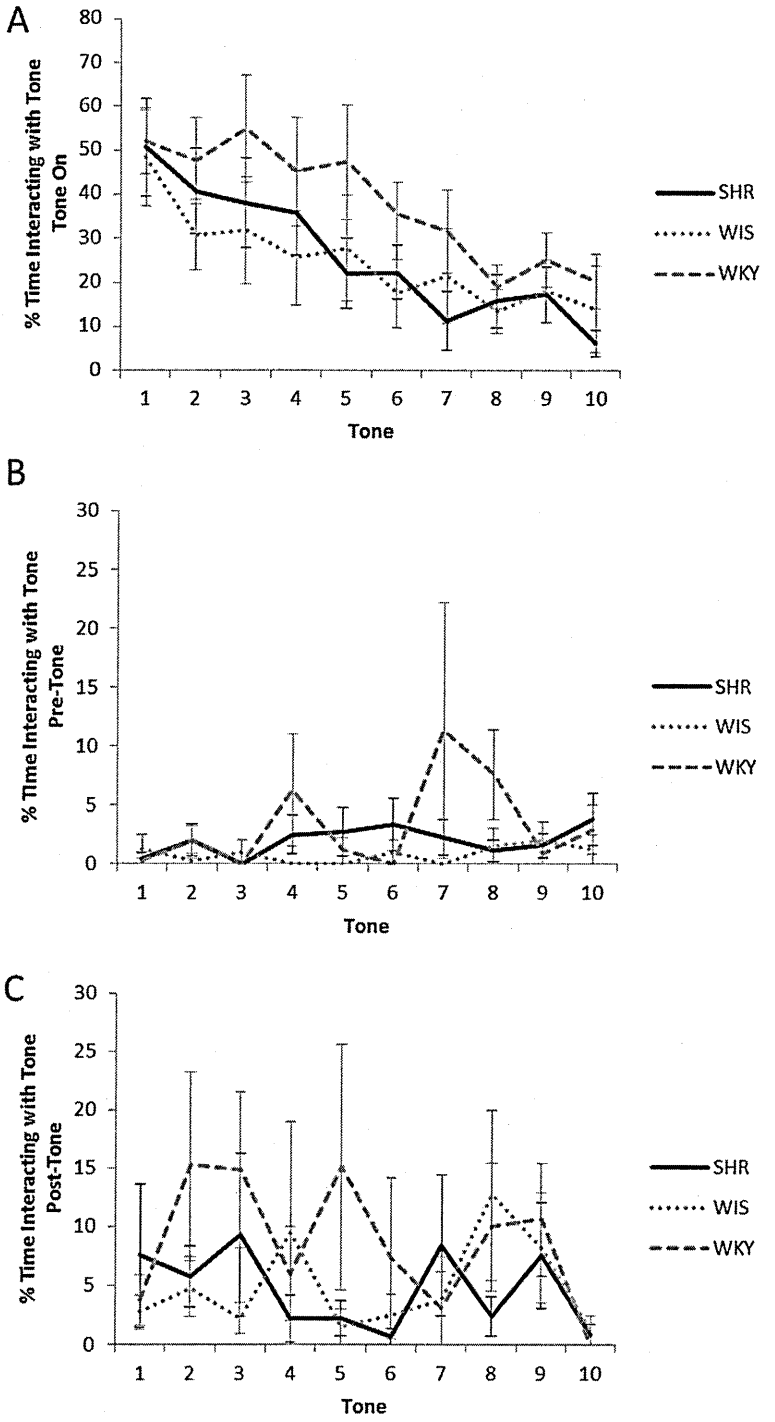

Figure 4.2: Graphs to show the time spent engaging with the auditory equipment over the 10 consecutive trials: A: 5-seconds while stimulus was on; B: 5-second pre-stimulus; C: 5-seconds post-stimulus. There was a significant main effect of stimulus presentation when the stimulus was on with animals reducing duration of response with increasing stimulus presentation. There were no significant main effects of stimulus presentation, strain or interactions for the time period pre- and post-stimulus onset.

In summary, behavioural testing revealed that the SHR responded to the stimulus with a similar frequency and duration to the two control strains. There were also no differences

between strains in responsiveness towards the stimulus object in the absence of the stimulus itself.

4.3.2. PHYSIOLOGICAL DIFFERENCES

Inclusion criteria

The recording positions (27 SHR; 28 WIS; 31 WKY) used in the data analysis were all in the deeper layers of the SC, as shown in the reconstruction of the sections in Figure 4.3 and tabulated in Table 4.2 and Table 4.3. Of the 86 auditory responses used for stimulus response analysis. 49 were positioned in the Intermediate Grey (InG; 14 SHR;, 15 WIS; 20 WKY), 3 were recorded from the Intermediate White (InW; 1 SHR; 1 WIS; 1 WKY), with the remaining 24 responses were recorded in the deep layers (Deep Grey, DpG; 10 SHR; 12 WIS; 10 WKY; Deep White DpWh; 2 SHR). Chi-square analysis showed there were no significant association between STRAIN and the positioning of the electrodes in terms of anterior-posterior positioning (χ^2=2.32; df=4; p=0.678); medial- lateral positioning (χ^2=0.29; df=2; p=0.864) or deeper layer positioning (χ^2=5.32; df=6;, p=0.504).

Co-ordinates From Bregma	Layer	SHR N=27	WIS N=28	WKY N=31
-5.8mm	Intermediate Grey	1	2	5
	Intermediate White	0	1	0
	Deep Grey	3	4	2
	Deep White	0	0	0
-6.3mm	Intermediate Grey	8	10	11
	Intermediate White	1	0	0
	Deep Grey	4	7	6
	Deep White	2	0	0
-6.8mm	Intermediate Grey	5	3	4
	Intermediate White	0	0	1
	Deep Grey	3	1	2
	Deep White	0	0	0

Table 4.2: The anterior-posterior and layer positioning of the electrodes for the auditory responses within the deeper layers of the superior colliculus for each strain. Chi-square analysis revealed no significant association.

	SHR	WIS	WKY
Medial Recordings	19	19	23
Lateral Recordings	8	9	8

Table 4.3: The medial-lateral positioning of the electrodes for the auditory responses. Chi-square analysis revealed no significant association.

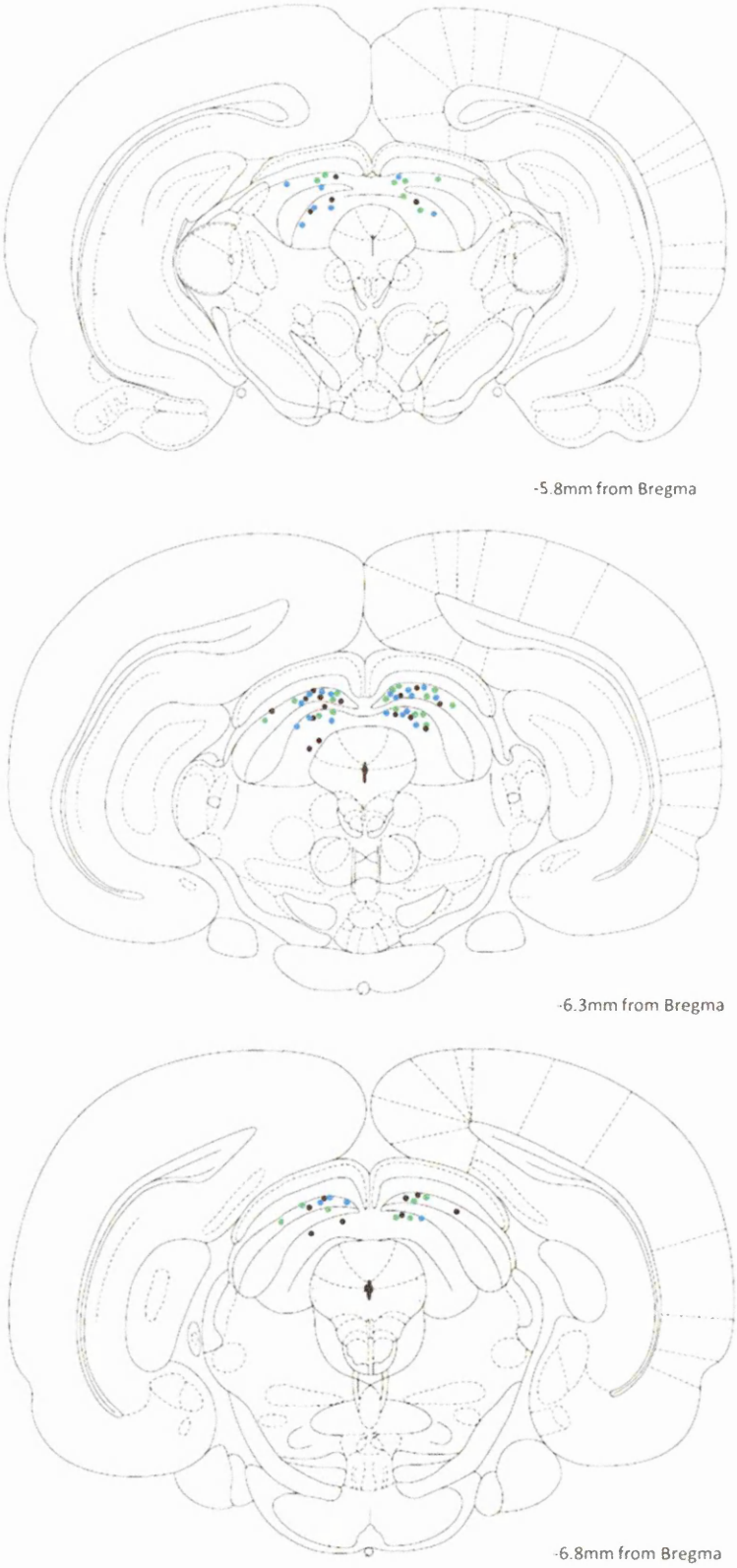

Figure 4.3: Reconstructed plots of recording sites in the SC. During collicular recordings, SHR recording sites are shown in black, WKY recording sites are shown in green, and Wistar recording sites are shown in blue. Adapted from Paxinos and Watson (1998).

4.3.2.1. AUDITORY STIMULATION LOCAL FIELD POTENTIALS

Stimulus- response relationship

Responses were recorded to five different intensities of an auditory stimulus as outlined in Section 2.3.4, an example of an auditory response LFP waveform average at the middle intensity of a stimulus-response curve for each strain is shown in Figure 4.4. A response was deemed to have occurred if the trace exceeded a pre-determined threshold after stimulus onset, as specified in Section 2.3.5. Based on these criteria not all animals responded to all stimulus intensities.

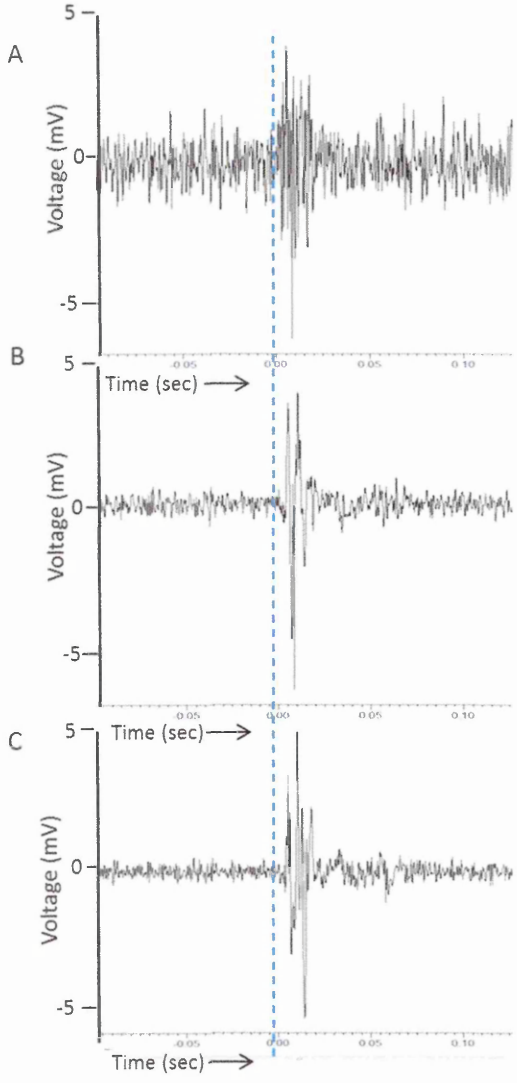

Figure 4.4: An example of an auditory response LFP waveform average at the middle intensity of a stimulus-response curve: A: SHR; B: WIS; C: WKY. The dotted blue line represents the point in time when the stimulus occurred.

The percentage of animals responding at each intensity is shown by strain in Table 4.3 and Figure 4.5, where stimulus intensity 55 dB SPL is the lowest intensity and stimulus intensity 75 dB SPL is the highest. Animals were least likely to respond at the lowest and second lowest intensities, where maximum responsiveness was 61.3 % and 89.3 % respectively. Chi-Square analysis revealed a significant association between strain and likelihood of responding at the 55 dB SPL intensity, where the SHR was significantly less likely to respond (see Table 4.4 for statistics). By the 60 dB SPL intensity the majority of animals were responding and again there were no differences in likelihood of responses between the strains.

Stimulus intensity (dB SPL)	Percentage of animals responding			Analysis of strain differences		
	SHR(n=27)	WIS (n=28)	WKY(n=31)	χ^2	df	p
55	25.9	53.6	61.3	7.81	2	0.020
60	81.5	89.3	85.5	2.08	2	0.354
65	96.3	92.9	87.1	1.69	2	0.430
70	100	96.4	96.8	0.95	2	0.624
75	100	100	100	N/A		

Table 4.4: The percentage of rats that responded at each auditory intensity, where 55 dB SPL is the lowest auditory intensity and 75 dB SPL is the maximum auditory intensity. There was a significant difference at the 55 dB SPL intensity where the SHR was significantly less likely to respond.

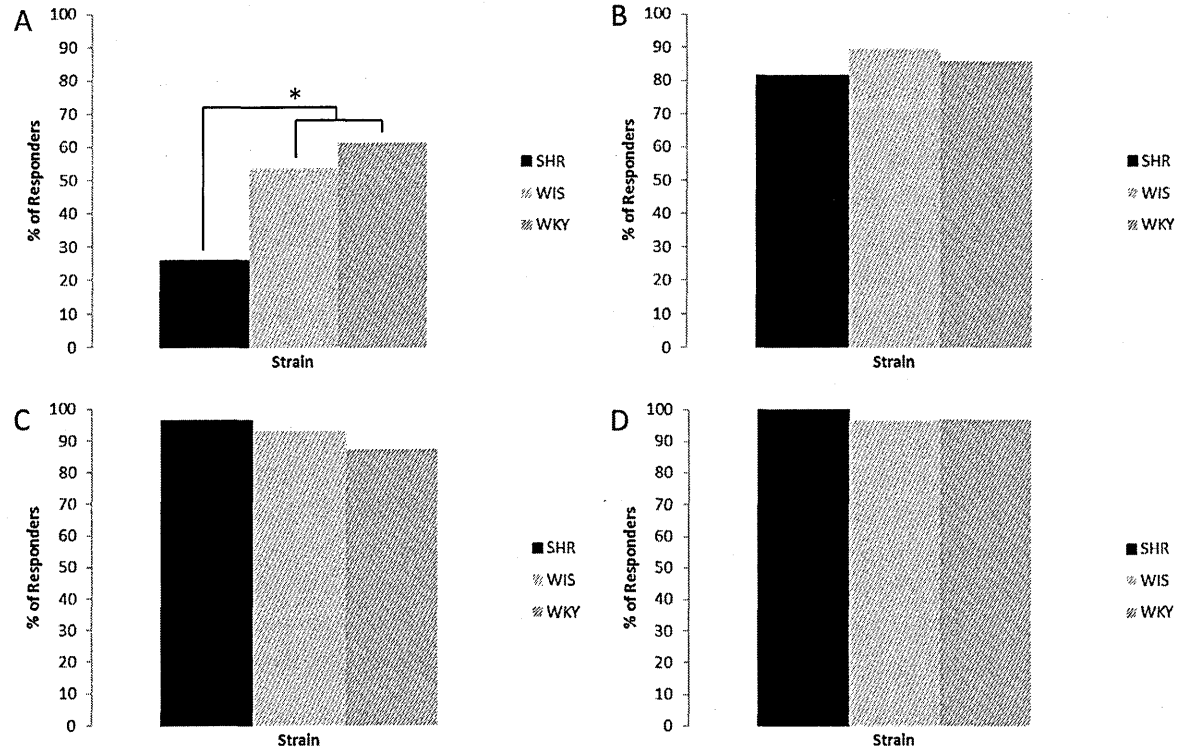

Figure 4.5: The percentage of rats that produced a LFP auditory response to the first four auditory levels; A: 55 dB SPL; B: 60 dB SPL; C: 65 dB SPL; D: 70 dB SPL. The SHR were significantly less likely to respond at the first intensity. (* $p<0.05$).

In order to look at effects of stimulus intensity, data from only the final three intensities were analysed, and hence only data from animals that responded to the final three intensities were used. For the 65 dB SPL auditory intensity, 1 SHR, 2 WIS and 4 WKY did not respond, so were excluded from the analysis of the response parameters (onset latency; peak-to-peak amplitude; duration; final N = SHR n=26; WIS n=26; WKY n=27). By the 70 dB SPL stimulus intensity, all animals responded other than 1 WIS and 1 WKY. All animals responded to the auditory stimulus at the 75 dB SPL intensity.

Onset latency

The onset latency for responses to stimulus intensities of 65-75 dB SPL is shown in Figure 4.6 as the mean ± SEM. There was a significant main effect of STIMULUS INTENSITY (F=5.44; df=2, 0.08; p=0.005). Within-subjects contrasts showed that there was a significant decrease in onset latency between the 65 dB SPL and 70 dB SPL stimulus intensity (F=10.24; df=1, 0.14; p=0.002). There was also a significant increase in onset latency between the 70 dB SPL and 75 dB SPL intensity (F=6.43; df=1; p=0.090), therefore there was no significant difference between the 65 dB SPL and 75 dB SPL stimulus intensity (F=0.14; df=1, 0.00; p=0.710). There was a trend towards a significant main effect of STRAIN (F=2.79; df=2, 0.08; p=0.069). Post hoc (Tukey HSD) analysis showed that the only difference nearing significance was SHR having a trend towards a significantly greater onset latency in comparison to the WKY only (p=0.055). There was no significant STIMULUS INTENSITY x STRAIN interaction (F=0.94; df=4, 0.03; p=0.442).

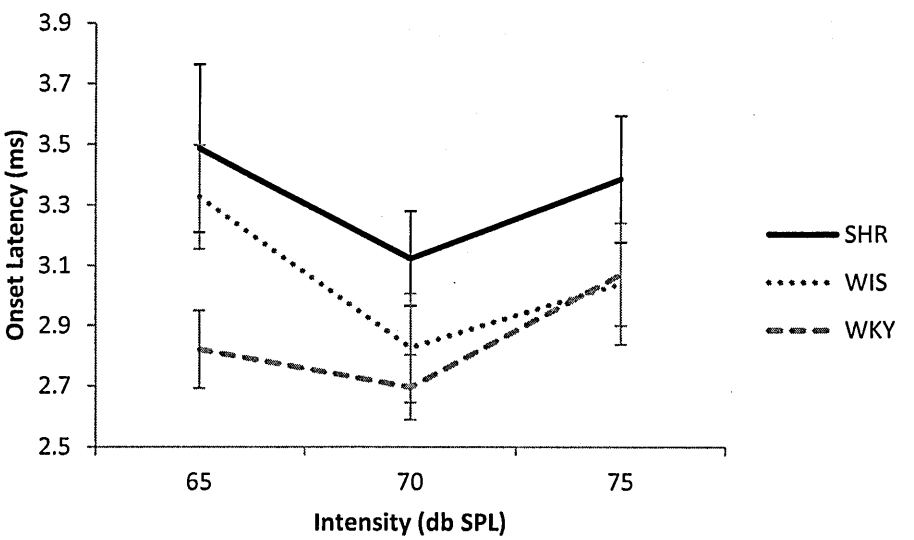

Figure 4.6: The LFP auditory response onset latency (mean ± SEM) of the three strains for the final three stimulus intensities. There was a significant main effect of STIMULUS INTENSITY with a significant decline in onset latency as the intensity increased between the 65 dB SPL and 70 dB SPL intensities, and a significant increase between the 70 dB SPL and 75 dB SPL intensity. There was a trend towards a main effect of STRAIN. The SHR had a trend towards significantly greater onset latency in comparison to the WKY. There was no significant interaction.

Peak-to-peak amplitude

The peak-to-peak amplitude for responses to stimulus intensities of 65-75 dB SPL is shown in Figure 4.7 as the mean ± SEM. There was a trend towards a significant main effect of STIMULUS INTENSITY (F=2.98; df=1.78, 0.04; p=0.061). Within-subjects contrasts showed that there was no significant difference in amplitude between the 65 dB SPL and 70 dB SPL stimulus intensity (F=2.49; df=1, 0.04; p=0.120), or between the 70 dB SPL and 75 dB SPL intensity (F=0.34; df=1; p=0.561), but there was a significant increase between the 65 dB SPL and 75 dB SPL stimulus intensity (F=7.58; df=2, 0.10; p=0.008). There was a significant main effect of STRAIN (F=10.60; df=2, 0.25; p=0.0005). Post hoc (Tukey HSD) tests showed that the SHR had a significantly lower amplitude in comparison to the WKY (p=0.0005) and a trend towards the SHR having a significantly lower amplitude than the WIS (p=0.051). Despite the WKY having a higher peak-to-peak amplitude across all intensities in comparison to the WIS, this was not significant (p=0.085). There was no significant STIMULUS INTENSITY x STRAIN interaction (F=1.20; df=3.55, 0.06; p=0.107).

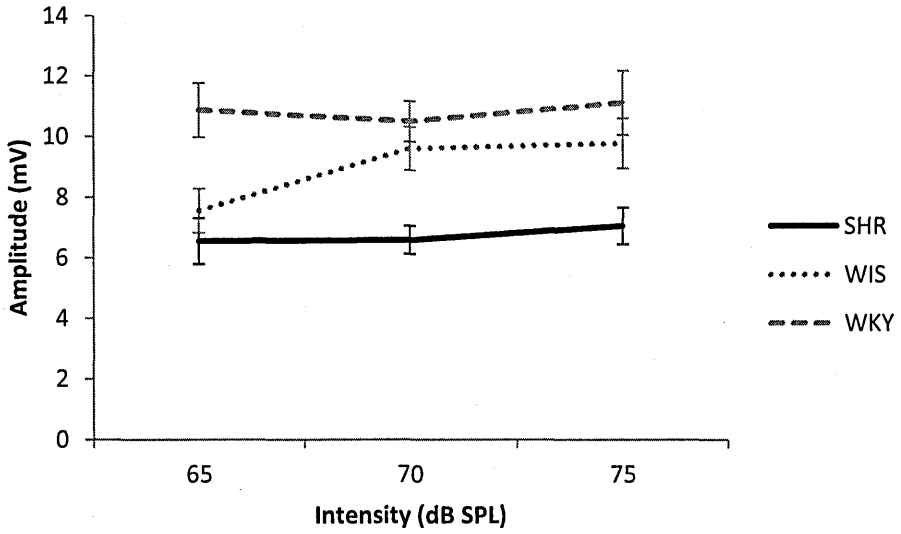

Figure 4.7: The LFP auditory response peak-to-peak amplitude (mean ± SEM) of the three strains for the final three stimulus intensities. There was a trend towards a significant main effect of STIMULUS INTENSITY, with a significant increase in peak-to-peak amplitude as the intensity increased between the 65 dB SPL and 75 dB SPL intensities. There was a main effect of STRAIN. The SHR had significantly lower amplitudes than the WKY and a trend towards significant than the WIS. There was no significant STIMULUS x STRAIN interaction.

Duration

The duration of responses to stimulus intensities of 65-75 dB SPL is shown in Figure 4.8 as the mean ± SEM. There was no significant main effect of STIMULUS INTENSITY (F=0.06; df=1.65, 0.001; p=0.918). There was no significant main effect of STRAIN (F=0.63; df=2, 0.02; p=0.534). There was a trend towards a significant STIMULUS INTENSITY x STRAIN interaction (F=2.61; df=3.30, 0.07; p=0.050). The graph indicates that this may be due to the SHR having a different overall pattern with an increase in duration to the 70 dB SPL tone whilst the other two strains show a decrease to this tone relative to the 65 and 75 dB SPL tones. This suggestion is supported by the lack of interaction found if a restricted repeated measures ANOVA is conducted removing the SHR (F=1.81; df=1.48, 0.03; p=0.178).

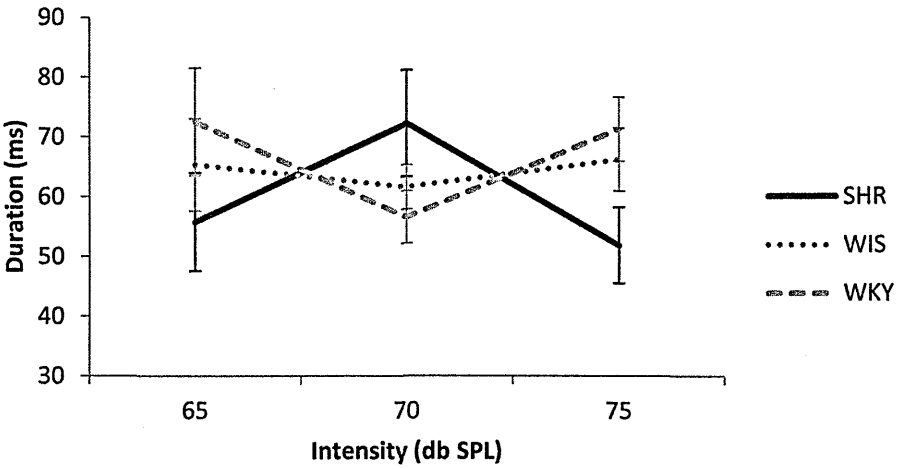

Figure 4.8: The LFP auditory response duration (mean ± SEM) of the three strains for the final three stimulus intensities. There was no significant main effect of STIMULUS INTENSITY or STRAIN. There was a trend towards a significant STIMULUS x STRAIN interaction likely caused by the SHR having a different response pattern to the other two strains.

In summary, LFP responses to auditory stimuli revealed that the SHR was least likely to respond at the lowest intensity. The SHR did differ in response amplitude and onset latency, having a trend towards significantly greater onset latency in comparison to one control strain. The SHR also had significantly lower amplitudes compared to the WKY and a trend towards a significant difference than the WIS. There were no significant differences between strains for response duration.

Stimulus – response relationship

Responses were recorded to five different intensities of an auditory stimulus, an example of an auditory response LFP waveform average at the middle intensity of a stimulus-response curve for each strain is shown in Figure 4.9. A response was deemed to have occurred if the level of activity rose above the upper threshold of the mean +1.96 SD, for at least 5 ms (5 consecutive bins). Based on these criteria, not all animals responded to all stimulus intensities.

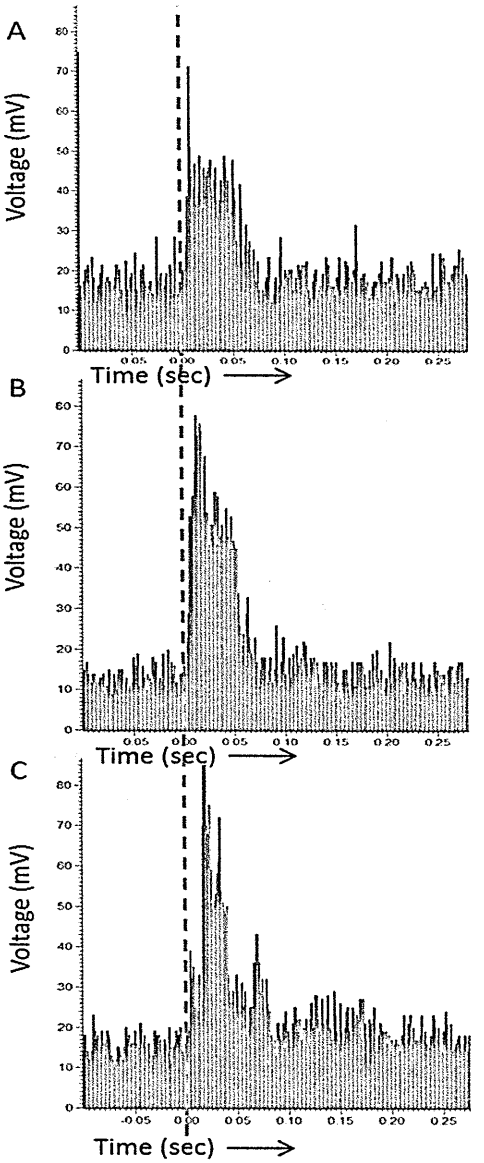

Figure 4.9: An example of an auditory response multiunit activity PSTH at the middle intensity of a stimulus-response curve: A: SHR; B: WIS; C: WKY. The dotted blue line represents the point in time when the stimulus occurred.

The percentage of animals responding at each intensity is shown by strain in Table 4.4 and Figure 4.10, where 55 dB SPL stimulus intensity is the lowest intensity and 75 dB SPL stimulus intensity is the highest. Animals were least likely to respond at the lowest and second lowest intensities where maximum responsiveness was 53.3 % and 80.6 % respectively. A Chi-Square analysis revealed a significant association between strain and likelihood of responding at the first intensity where the WKY was most likely to respond (see Table 4.5 for statistics). By the 65 dB SPL intensity the majority of animals were responding and again there were no differences in likelihood of responses between the strains.

Stimulus intensity (dB SPL)	Percentage of animals responding			Analysis of strain differences		
	SHR(n=27)	WIS (n=28)	WKY(n=31)	χ^2	df	p
55	20.0	28.6	53.3	6.22	2	0.045
60	70.4	60.7	80.6	2.84	2	0.242
65	96.3	92.9	87.1	1.69	2	0.430
70	100	92.9	96.8	2.09	2	0.351
75	100	100	100	N/A		

Table 4.5: The percentage of rats that responded at each auditory intensity, where 55 dB SPL is the lowest auditory intensity and 75 dB SPL is the maximum auditory intensity. The WKY was significantly more likely to respond than the SHR or WIS at the 55 dB SPL intensity.

In order to look at effects of stimulus intensity, the final three intensities were analysed. For the 65 dB SPL auditory intensity, 1 SHR, 2 WIS and 4 WKY did not respond, so were excluded from the analysis of the response parameters (onset latency; peak-to-peak amplitude; duration; final N= SHR n=26; WIS n=26; WKY n=27). By the 70 dB SPL stimulus intensity, all animals responded other than 2 WIS and 1 WKY; all animals responded to the auditory stimulus at the 75 dB SPL intensity.

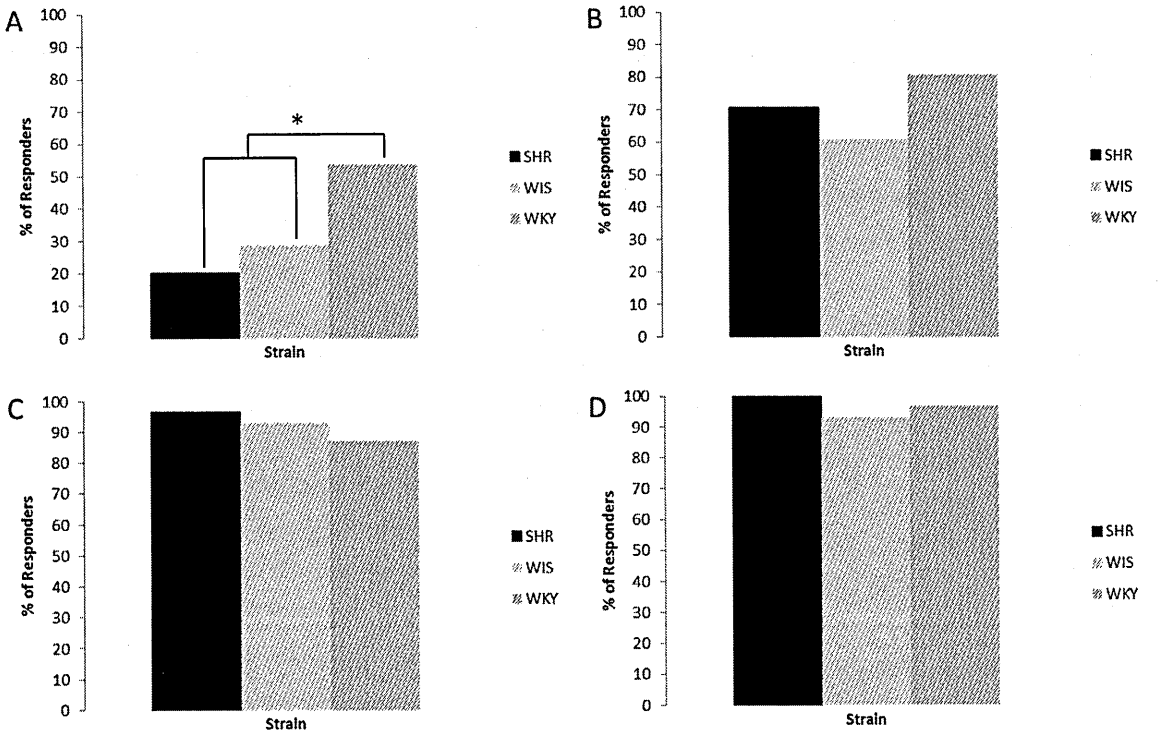

Figure 4.10: The percentage of rats that produced a multiunit activity auditory response to the lowest two auditory levels; A: 55 dB SPL; B: 60 dB SPL; C: 65 dB SPL; D: 70 dB SPL. The WKY was significantly more likely to respond than the two control strains at the lowest auditory intensity.

Onset latency

The onset latency for responses to stimulus intensities of 65-75 dB SPL is shown in Figure 4.11 as the mean ± SEM. There was a significant main effect of STIMULUS INTENSITY ($F=6.56$; df=1.55, 0.09; $p=0.004$). Within-subjects contrasts showed that there was a significant decrease in onset latency between the 65 and 70 dB SPL stimulus intensity ($F=4.97$; df=1, 0.07; $p=0.029$), and 65 dB SPL and 75 dB SPL intensity ($F=9.13$; df=1, 0.12; $p=0.004$). There was no significant difference between the 70 dB SPL and 75 dB SPL intensity ($F=2.91$; df=1; $p=0.093$). There was a significant main effect of STRAIN ($F=5.93$; df=2, 0.15; $p=0.004$). Post hoc (Tukey HSD) tests showed that the SHR had a significantly greater onset latency in comparison to the WKY ($p=0.006$) and the WIS ($p=0.020$). There was also a significant STIMULUS INTENSITY x STRAIN interactions ($F=3.23$; df=3.11, 0.09; $p=0.024$). Restricted ANOVAs revealed that the SHR had a decrease in onset latency as the intensities increased in comparison to WIS and WKY, with comparable onset latency times

for the final highest intensity. One-Way ANOVAs revealed at 65 dB SPL intensity the SHR had a significantly greater onset latency than the WKY (F=5.11; df=1; p=0.029), with a trend towards significance with the WIS (F=3.64; df=1; p=0.063), and at the 70 dB SPL intensity the SHR had a significantly greater onset latency than the WIS (F=7.26; df=1; p=0.010) and a trend towards significance for the WKY (F=3.61; df=1; p=0.064). There was a significant interaction between the SHR and WIS (F=5.99; df=1, 0.13; p=0.019) and WIS and WKY (F=7.12; df=1, 0.14; p=0.011) between the 70 dB SPL and 75 dB SPL intensity, where the WIS showed an increase in onset latency, yet both the SHR and WKY had a decrease in onset latency. No strain differences by the final intensity.

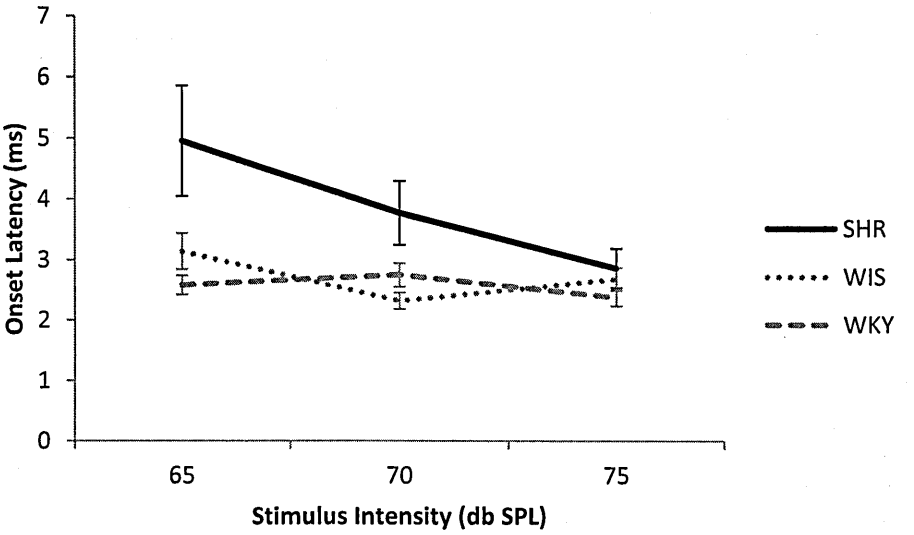

Figure 4.11: The multiunit activity auditory response onset latency (mean ± SEM) of the three strains for the final three stimulus intensities. There was a significant main effect of STIMULUS INTENSITY, as the intensity increased, the onset latency decreased. There was a main effect of STRAIN with the SHR having significantly greater onset latency than the WKY and the WIS. There was a significant STIMULUS x STRAIN interaction, where the SHR had a dramatic decline in response onset latency in comparison to the two control strains, such that there were significant strain differences at the 65 dB SPL and 70 dB SPL intensity, yet not at the 75 dB SPL intensity. Between the 70 dB SPL and 75 dB SPL intensity, the WIS showed an increase in onset latency, yet both the SHR and WKY had a decrease in onset latency.

Amplitude

The amplitude for responses to stimulus intensities of 65-75 dB SPL is shown in Figure 4.12 as the mean ± SEM. There was a significant main effect of STIMULUS INTENSITY (F=6.25; df=2, 0.09; p=0.003). Within-subjects contrasts showed that there was a significant increase in amplitude between the 65 dB SPL and 70 dB SPL stimulus intensity (F=4.62; df=1, 0.7; p=0.035), and 65 dB SPL and 75 dB SPL intensity (F=10.52; df=1, 0.14; p=0.002). There was no significant difference between the 70 dB SPL and 75 dB SPL intensity (F=0.39; df=1; p=0.536). There was no significant main effect of STRAIN (F=2.27; df=2, 0.07; p=0.11) or significant STIMULUS INTENSITY x STRAIN interaction (F=0.71; df=4, 0.02; p=0.585) for this parameter.

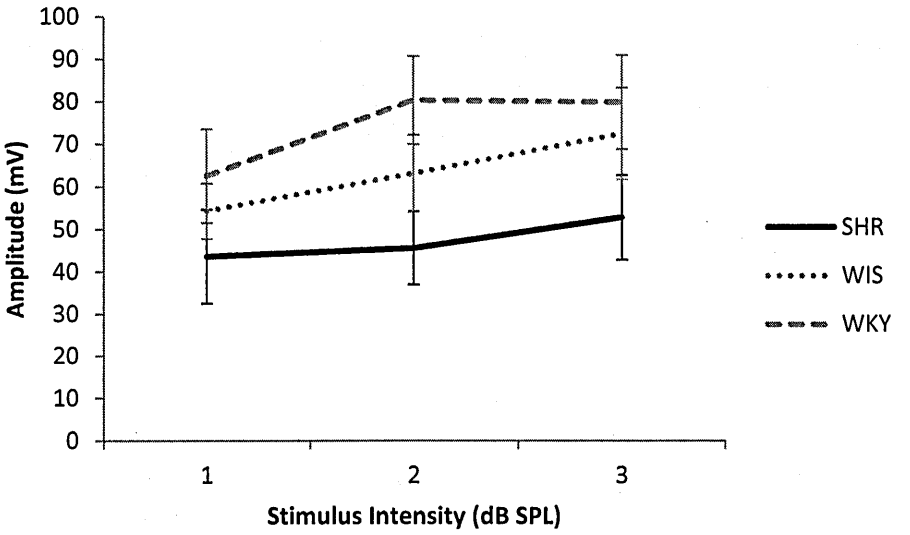

Figure 4.12: The multiunit activity auditory response amplitude (mean ± SEM) of the three strains for the final three stimulus intensities. There was a significant main effect of STIMULUS INTENSITY, as the intensity increased, the amplitude increased. There was no significant main effect of STRAIN or significant STIMULUS x STRAIN interaction for this parameter.

Duration

The duration for responses to stimulus intensities of 65-75 dB SPL is shown in Figure 4.13 as the mean ± SEM. There was a significant main effect of STIMULUS INTENSITY (F=3.20; df=2, 0.047; p=0.044). Within-subjects contrasts showed that there was a significant increase in duration between the 65 dB SPL and 70 dB SPL stimulus intensity (F=0.62; df=1, 0.01; p=0.043) and the 65 dB SPL and 75 dB SPL stimulus (F=8.26; df=1, 0.11; p=0.005). There was no significant difference between the 70 dB SPL and 75 dB SPL intensity (F=1.47; df=1; p=0.228). There was no main effect of STRAIN (F=0.60; df=2, 0.018; p=0.55) or STIMULUS INTENSITY x STRAIN (F=2.16; df=2, 0.062; p=0.077), indicating that all strains responded in a comparable manner to increasing stimulus intensity.

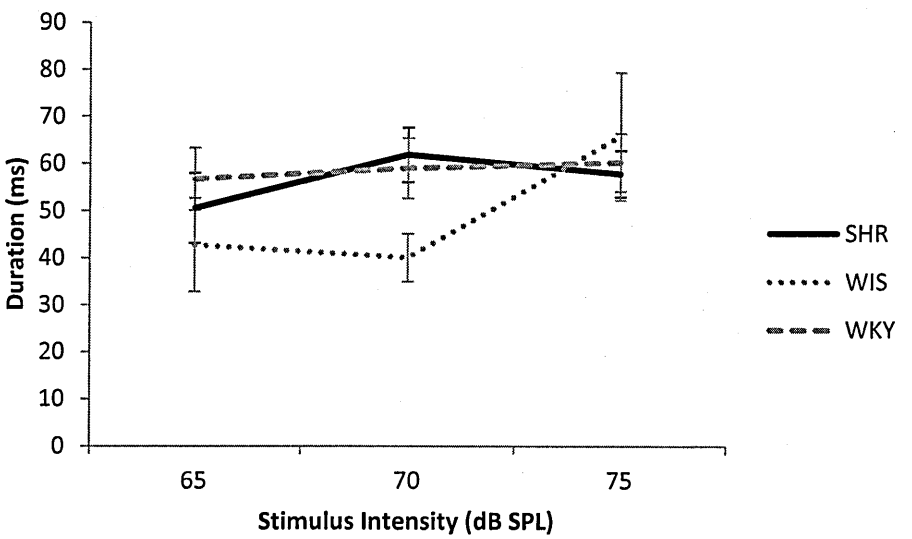

Figure 4.13: The multiunit activity auditory response duration (mean ± SEM) of the three strains for the final three stimulus intensities. There was a significant main effect of STIMULUS INTENSITY. As the intensity increased the duration increased. There was no significant main effect of STRAIN or significant STIMULUS x STRAIN interaction for this parameter.

In summary, multiunit activity responses to auditory stimuli revealed the SHR was less likely to respond at the 55 dB SPL intensity in comparison to the WKY strain only. The WKY was also significantly more likely to respond than the WIS at the first intensity. The SHR had a

significantly greater onset latency than the two control strains, but there was a significant interaction also. The SHR had a dramatic decline in response onset latency in comparison to the two control strains; such that there were significant strain differences at the 65 dB SPL and 70 dB SPL intensity, yet not at the 75 dB SPL intensity. Between the 70 dB SPL and 75 dB SPL intensity, the SHR responded similarly to the WKY with a decrease in onset latency, while the WIS showed an increase in onset latency.

4.3.3. VOLUME ESTIMATES AND FRACTION

Volume estimates

The volume of the deeper layers of the colliculus was estimated using the Cavalieri method as described in Chapter 2 (Section 2.4.2) and is shown in Figure 4.14a. A strain comparison was made using a One-Way ANOVA revealed a significant difference between the three strains ($F=5.36$; $df=2$; $p=0.017$). The SHR had the smallest estimated volume (1.76 ± 0.07 $x10^{10}$ μm^3) in comparison to the WIS (2.19 ± 0.13 $x10^{10}$ μm^3) and WKY (2.27 ± 0.14 $x10^{10}$ μm^3). Post hoc (Tukey HSD) tests showed that the SHR had significantly smaller collicular volumes than the WKY ($p=0.020$), and a trend towards a significant difference compared to the WIS ($p=0.055$).

As shown previously (Chapter 3), a strain comparison using a One-Way ANOVA for whole brain volume revealed a significant difference between the three strains ($F=3.86$; $df=2$; $p=0.044$; Figure 4.14b). Post hoc (Tukey HSD) tests revealed that the SHR had a significantly smaller whole brain volume than the WIS ($p=0.041$) but not the WKY ($p=0.172$). There was no significant difference between the WIS and WKY ($p=0.707$). Given these differences in whole brain volume, it was necessary to normalise collicular volume to whole brain volume, and therefore calculate a volume fraction. The results of this are considered in the next section.

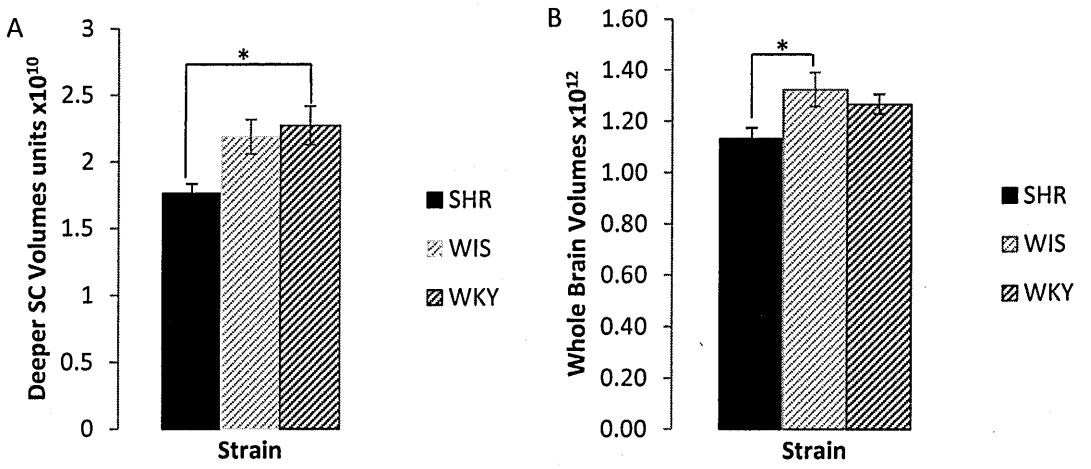

Figure 4.14: The estimated volumes of the areas analysed in the three strains for A: deep layers of the colliculus, B: whole brain volume. The SHR had significantly smaller brain volume than the WIS, and significantly smaller deeper SC layers than both control strains. (* $p<0.05$).

Volume fraction

When the proportion of the SC to the whole brain volume, the volume fraction, was compared between strains using a One-Way ANOVA there was no significant difference ($F=1.00$; $df=2$; $p=0.391$, see Figure 4.15). The deep layers of the SC were 1.58±0.12% of the SHR total brain volume, similar to the WIS and WKY with a volume fraction of 1.66±0.07% and 1.81±0.16% respectively (see Figure 4.15).

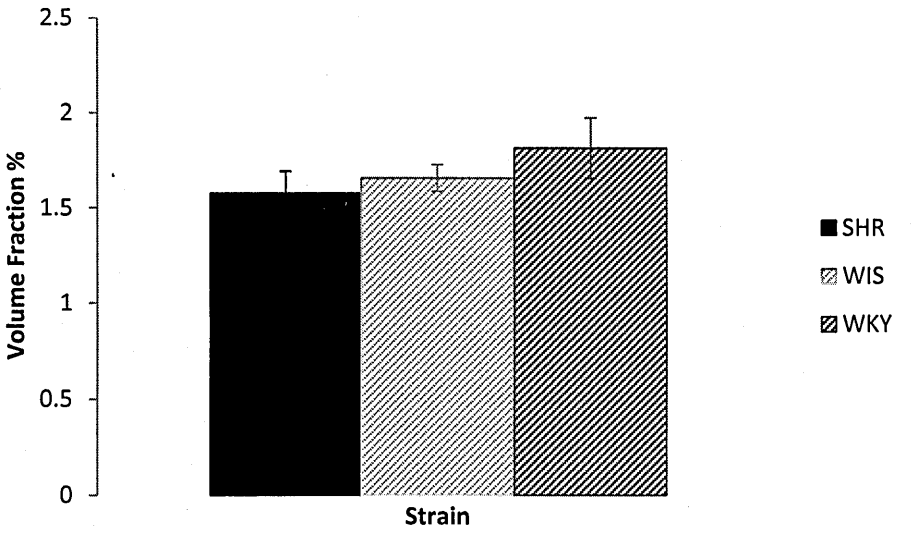

Figure 4.15: There were no significant differences in the volume fraction of the SC deep layers for all three strains.

4.3.4. CELL COUNTS AND DENSITY

Cell counts

Cell counts in the intermediate layers were measured as described in in Chapter 2 (Section 2.4.3) and are shown in Figure 4.16. A strain comparison made using a One-Way ANOVA revealed a significant difference between the three strains in the amount of neurons ($F=4.89$; $df=2$; $p=0.025$) and glia ($F=3.73$; $df=2$; $p=0.050$) and therefore total cell counts ($F=5.29$; $df=2$; $p=0.019$) in the deeper layers. Post hoc (Tukey HSD) tests revealed that the SHR had significantly less neurons than the WKY ($p=0.029$), but not the WIS ($p=0.926$), there was no significant difference between the WIS and WKY ($p=0.75$). Similarly, post hoc (Tukey HSD) tests revealed that the SHR had significantly less glia than the WKY ($p=0.041$), but not the WIS ($p=0.360$), there was no significant difference between the WIS and WKY ($p=0.480$). Therefore, post hoc (Tukey HSD) tests revealed that the SHR had significantly less total cell counts than the WKY ($p=0.016$), but not the WIS ($p=0.541$), there was no significant difference between the WIS and WKY ($p=0.153$).

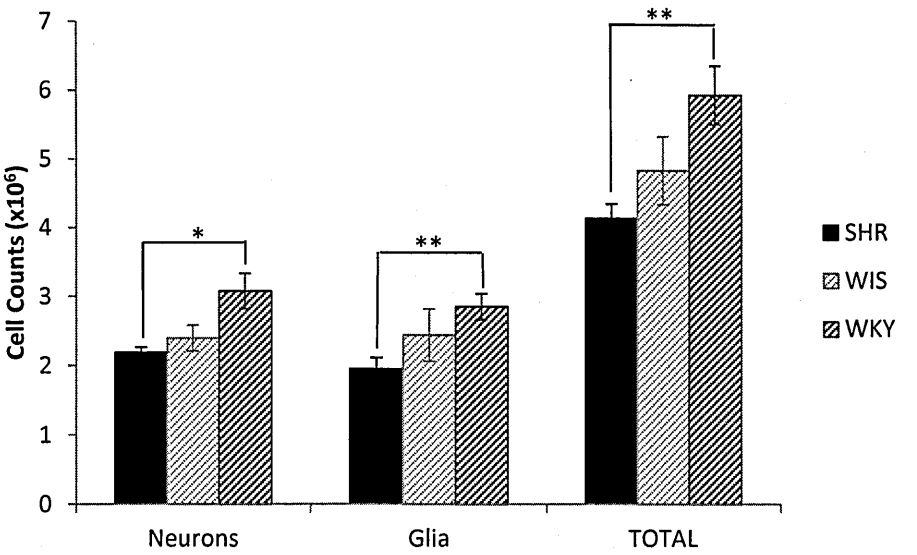

Figure 4.16: The estimated cell counts, of neurons, glia and total cell counts within the deep layers of the SC of the three strains; the SHR had significantly less neurons, glia and total cell counts in comparison to the WKY only. (* $p<0.05$; ** $p<0.005$).

Neuron: glia ratio

There were no significant differences in the ratio of neurons: glia when the strains were compared using a One-Way ANOVA ($F=0.84$; $df=2$; $p=0.451$, see Figure 4.17).

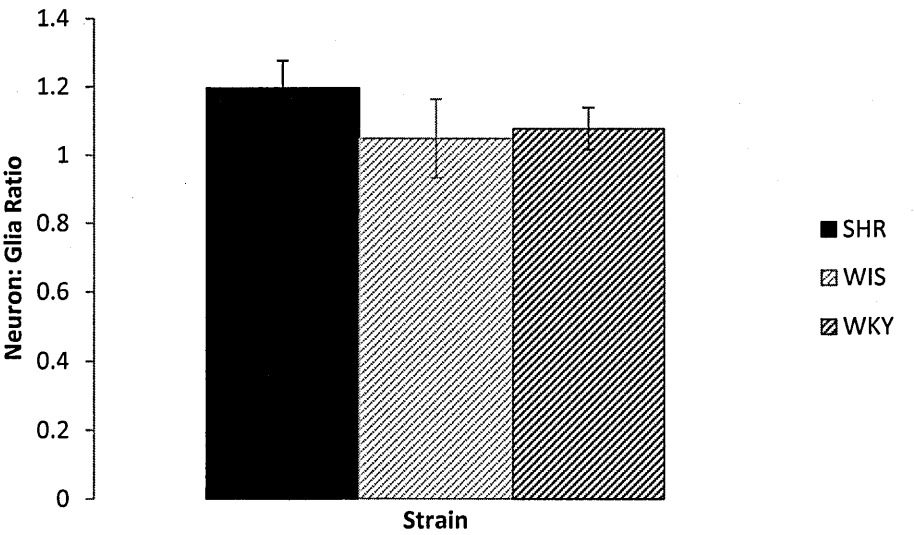

Figure 4.17: Neurons: Glia ratio in the deep layers. There were no significant differences found.

Cell density

Cell densities in the deeper layers were measured as described in Chapter 2 (Section 2.4.3) and are shown in Figure 4.18. A strain comparison made using a One-Way ANOVA revealed no significant differences between the three strains in neurons (F=0.52; df=2; p=0.608); glia (F=0.12; df=2; p=0.892) or total cell densities (F=0.01; df=2; p=0.990).

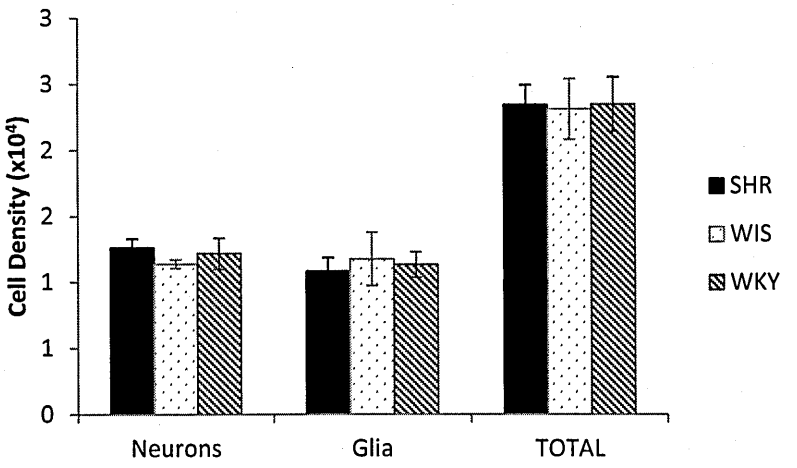

Figure 4.18: The cell density of neurons, glia and all cells in the deeper layers; there were no significant differences found.

In summary, the morphological analysis of the deep layers of the colliculus revealed that the SHR had significantly smaller brain volumes but this difference was only found with reference to one of the control strains. The SHR had significantly smaller deeper SC layers than both control strains but the SHR did not differ to the two control strains in the volume fraction of the SC deeper layers for all three strains. The SHR had significantly less neurons, glia and total cell counts in comparison to one control strain, but the SHR did not differ to the two control strains in cell densities.

4.4. DISCUSSION

It was hypothesised that performance on a collicular-dependent behavioural task would differ between the SHR and control strains. In order to ensure the task was dependent on the deeper SC, this was tested using an auditory stimulus. Behavioural testing revealed that all strains responded to the stimulus in a similar manner for all 10 trials. There was a trend towards a significant main effect of strain with WKY spending more time with the stimulus

than the SHR and WIS, but post hoc analysis revealed no significant strain differences. These are different findings to the behavioural engagement with the light stimulus (see Section 3.3.1) where it was the SHR that showed longer duration responses and also reduced habituation.

Secondly, it was hypothesised that there would be significant differences in the physiological responses to sensory stimuli between the SHR and control strains. As with the behavioural testing, this was investigated by recording responses to auditory stimuli, in the anaesthetised rat (Section 2.3). In the present electrophysiological study, as to be expected, an increase in auditory stimulus intensity caused an increase in both LFP (increase in amplitude), and multiunit (decrease in onset latency, increase in amplitude and duration) auditory responses. The SHR was least likely to produce an LFP response to the lowest auditory intensity relative to both control strains. The SHR was also least likely to produce a multiunit response to the lowest intensity but only by comparison to the WKY; it did not differ from the WIS. This differs from the vision data where there was no difference in LFP response likelihood and for multiunit responses the SHR was most likely to respond (see Section 3.3.2).

The LFP data, which can be considered to represent inputs to the SC, showed a trend towards the SHR having greater onset latency in comparison to the WKY. There was also a reduction in amplitude in the SHR relative to the WKY, which did reach significance and a reduction relative to the WIS which was not quite significant. There were no strain differences in duration. This is quite different to the responses to visual stimuli recorded from superficial layers which showed no difference between strains for latency or amplitude and a slight reduction in duration with the SHR showing a decrease relative to just one of the control strains. These strain differences in LFP data for auditory responses indicate that the SHR is less responsive to auditory stimuli. However, the fact that these

differences are found in the LFP (input) responses suggests they are driven by strain differences upstream of SC processing.

By contrast to the LFP data, for the multiunit responses to auditory stimuli there were no significant strain differences for amplitude. The lack of difference in amplitude indicates that the SC had in some way normalised the reduced input observed in the LFP responses. This can also be seen to some extent in the onset latency data. The multiunit responses data revealed that SHR did still show greater onset latency, however, there was also an interaction between STRAIN and STIMULUS INTENSITY which indicated that for the highest stimulus intensity, the onset latency had been normalised to the level of the two control strains. This too can be interpreted to mean that the collicular processing compensated for the altered incoming information, speeding up the latency, albeit only for the more intense stimuli. This could indicate that the processing within the SC of sensory information can be dependent on stimulus intensity. In any event, the reduced differences between the SHR and other strains in the multiunit data could indicate that the SC is hyper-responsive (similar to the finding of the visual response data), in the SHR, allowing it to compensate for this reduced response input, thus causing no significant differences in multiunit activity response outputs or behaviour; responses and behaviours are normalised.

Finally, it was hypothesised that there would be significant differences in morphological parameters of the SC (neuronal and glia cell counts and density) between the SHR and control strains. The structural analysis revealed that the SHR had significantly smaller brains than the WIS, and significantly smaller deeper SC layers than both control strains, yet there were no significant differences in the volume fraction of the SC deep layers for all three strains. The SHR had significantly less neurons, glia and total cell counts in

comparison to the WKY only, yet the discrepancy in brain volume must be taken into account when looking at absolute cell numbers and there were no significant strain differences in neuron: glia ratio or cell densities.

It has been shown that the external cortex of the inferior colliculus (IC) constitutes one, if not the most important source, of auditory information to the SC, and appears to contribute considerably to acoustico-motor pathways involved in SC-mediated orienting behaviour. Stimulation of the IC activates auditory superior collicular neurons, eliciting simultaneous movement of the ear in conjunction with contralaterally directed eye movements (Syka and Straschill, 1970; Huffman and Henson, 1990, for review). In comparison to the behavioural data using a visual stimulus, the animals did not habituate as quickly or significantly differently by strain to the auditory stimulus over the consecutive trials. This may be due to the tone being an unseen threat to the animals. It was not physically seen like the light flash, so may affect the amount of presentations needed to habituate to the non-novel stimuli. Similarly, the tone may have been classed as a more intense stimulus at 75dB SPL. A more intense stimulus will evoke a longer time period to habituate to it, especially as the inferior colliculus-SC pathway plays a crucial role in the generation of aversive and/or defensive motor commands (Dean et al., 1989). The ascending auditory inputs are denser than the ascending visual processing pathways in the SC, such that visual responses are far more depressed by cortical deactivation than are somatosensory, and somatosensory responses are far more depressed than are auditory (Stein and Meredith, 1993). The greater role of ascending inputs on auditory stimuli may also explain the differences between the auditory and visual behaviour task as this defensive inferior colliculus-SC pathway is a more crucial component of behavioural outcomes to auditory stimuli, than ascending pathways for visual processing.

Behaviourally, the WKY spent more time engaging with the auditory stimulus throughout the consecutive trials, although this did not quite reach significance and all strains responded to the stimulus at a similar rate as the 10 trials continued. The differences found could be due to the WKY being a more anxious strain (Langen and Dost, 2010). An informal observation during behavioural video analysis supports this because the WKY did spend more time frozen, staring at the object and more time in the outer circle of the arena in comparison to the WIS and SHR. Similar findings have been shown in the elevated plus maze, a measure of anxiety in these animals (Langen and Dost, 2010), where the WKY spent more time in the central compartment, rather than at the end of the arms. Similarly, McAuley et al. (2009) found the WKY to take a longer period of time to enter the central arena in an open field, as well as exhibiting less rearing behaviour. It has been suggested that the WKY have a hypervigilant state that may contribute to the anxiety vulnerability seen in this strain (McAuley et al., 2009). Interestingly, the SHR showed least amount of anxiety-related behaviour in the elevated plus maze in comparison to the WKY and Sprague Dawley. This effect was magnified with age, where the SHR showed a decreased 'anxiety' when compared to the two strains as the age increased (Ferguson and Gray, 2005).

The inferior colliculus - SC pathway plays a crucial role in the generation of aversive and/or defensive motor commands. Morphologically, inferior collicular inputs are densest within the caudo-medial quadrant of the SC. This quadrant mediates eye and head orientation to the upper visual field (Tiao and Blakemore, 1976), and the organisation of behavioural escape responses in rodents crucially depends on this pathway (Cohen and Castro-Alamancos, 2010). Stimulation of the tectopontine bundle, an uncrossed descending pathway arising from the SC produces avoidance behaviour in rats (Sahibzada et al., 1986). Inputs from the inferior colliculus are densest in this SC region (Redgrave et al., 1987; 1988). Electrical or chemical stimulation of the IC induces fear-like reactions such as freezing, fight, or wild running (Cardoso et al., 1994; Pandossio and Brandão, 1999).

Physiologically, the WKY strain was significantly more likely to produce multiunit activity responses at the lowest intensity, and the SHR was significantly less likely to produce an LFP response at the first intensity in comparison to the WKY. These findings (and as the SHR had significantly lower LFP amplitudes than the WKY and a trend towards significance vs WIS) suggest that the SHR had a lower responsiveness to auditory stimuli in the SC than the WKY, and potentially the WIS. The differences in LFP (input) responses indicate these strain differences are found prior to SC processing, such as a disruption or hypo-response to auditory stimulation through this inferior colliculus - SC pathway in the SHR, while arguably a potential hyper-responsive inferior colliculus - SC pathway in the WKY. Supporting this theory is that the WKY has been shown to have an enhanced startle response in comparison to Sprague-Dawley rats (McAuley et al., 2009), while the SHR has been found to have significantly lower startle amplitude that the WKY, and Sprague-Dawley rats, with no difference in startle habituation (Van den Buuse et al., 2004). It is worth noting here that the WKY is not a 'normal' control, and hence is the reason why the WIS was also used throughout the current experiments. As the WKY was significantly more likely to produce a multiunit activity response at the first intensity, it suggests that this difference in LFP (input) response has not been corrected within the SC processing, and will lead to a greater hypervigilance to low intensity auditory stimuli.

As mentioned in Section 1.3.2.2, the SHR develops hypertension with age. It has been shown that hypertension is an important pathophysiological risk factor in age-related hearing loss (Rarey et al., 1996), with deterioration in high-frequency (12- 24kHz) hearing sensitivity occurring in aged (18 months) hypertensive rats (Borg et al., 1982). Yet, as behaviourally there were no differences in auditory stimuli engagement and habituation between the SHR and WIS strain, or any responsiveness differences at the lower tone intensity, it would suggest that this is not the case with the animals in this study. Similarly the animals used in

this study were not as aged as in Borg et al. (1982) study, and a lower (8 kHz) frequency was assessed.

A study in the development of synaptic transmission in the retinocollicular pathway of the rat found a quickening of onset latencies as this system developed (Reece and Lim, 1988). These finding could suggest that the SHR has a development delay of this system, and may lead to the greater onset latencies seen in these animals. A quickening of onset latency is similarly found in the visual system as it develops in humans (Crognale et al., 2001). Significantly longer saccade latencies and duration in visually guided saccades (VGS, Mahone et al., 2009; Goto et al., 2010), memory guided saccades (MGS, Goto et al., 2010), prosaccades (Klein et al., 2003; Munoz et al., 2003), and antisaccades (Munoz et al., 2003; Feifel et al., 2004; Karatekin, 2006; Karatekin et al., 2010) has been shown in children with ADHD in occulomotor paradigms, a model which is highly dependent on the SC. ADHD is a developmental disorder, with childhood onset (see Section 1.1.1). The delay in onset latency was only seen for the weak stimuli, at the higher intensites all strains showed similar onset latency, suggesting that the delay in onset latency is only for weak stimuli that potentially others without ADHD would ignore. Therefore a developmental delay of the SC leading to deficient SC processing could link the longer onset latencies in the SC of the SHR and the saccade latencies seen in children with ADHD as well as the behavioural deficits seen in the SHR and children with ADHD.

In the present study, the SHR was shown to have significantly greater onset latency for both LFP and multiunit activity auditory responses. This is also a similar finding within the superficial layer visual responses. It suggests that this finding is due to a lack of development within the SC rather than upstream of it in these animals. Interestingly, Wallace et al. (1996) found similar findings in the new-born monkey, where SC neurons had

increased response latencies, as well as approximately half the occurrence of multisensory neurons in comparison to adulthood in the deeper layers of the SC. In other areas of the brain, this speeding of responses latencies occurs with development, Sonntag et al. (2009) found a gradual shortening in response latencies up to P18 in mice in regards to the development of sound-evoked discharge activity in the medial nucleus of the trapezoid body. This finding could suggest that the SHR has an incorrect development of this system, and may lead to the greater onset latencies seen in these animals; an interesting finding as ADHD is a developmental disorder. As previously mentioned, oculomotor paradigms, which are highly dependent on the SC frequently show children with ADHD having significantly longer saccade latencies (Mahone et al., 2009; Goto et al., 2010; Klein et al., 2003; Munoz et al., 2003). Similarly, children who received a cochlear implant, showed a significant decrease in minimum latency in auditory brainstem auditory-evoked potentials within the first year of implant use; underlying mechanisms to produce this plasticity were likely due to improvement in synaptic efficacy and possible increased myelination (Gordon et al., 2003).

Comparable findings were seen in the present morphological study to Chapter 3, where the SHR had significantly smaller total brain volume (than the WIS) and thus, smaller deeper colliculi than the two control stains. As previously mentioned, similar findings, where the SHR had smaller total brain volumes in comparison to the WKY have been reported (Nelson et al., 1993). A reduction in whole brain volume has been shown in the brains of individuals with ADHD (Castellanos et al., 2002), supporting the construct validity of the SHR. It is worth noting here that there was no difference in body weight of strain in the morphological study, so a reduction in brain volume was not due to a reduction in general body size. In the present study, the SHR were also found to have significantly less neurons, glia, and therefore total absolute cell counts compared to the WKY. However, given the significant differences in brain volume seen in these different strains, cell density provides

a more meaningful measure and this showed no significant differences. This indicates that the physiological differences found in the deeper layers of the SC in the three strains must be due to either differences in receptor densities, differences in the number of different types of neuron, i.e. a greater number of excitatory inputs compared to inhibitory ones; or differences in auditory structures upstream of the superior colliculus, i.e. the inferior colliculus.

5. AMPHETAMINE EFFECTS ON COLLICULAR RESPONSES

This chapter describes the findings from an investigation into the effects of intravenously administered amphetamine on visual superficial layer and auditory deeper layer collicular responses. As stated in the Introduction (Chapter 1), problems within the SC may underlie some of the symptoms found in ADHD and therefore the SC could be investigated in the SHR model of the condition. The key results of an investigation into visual responses in the SHR revealed that the SC may be hyper-responsive to stimuli. Behaviourally this manifests as longer duration responses and reduced habituation to visual stimuli. It is suggested that the animal has less ability to assess the saliency of, and habituate to, non-salient stimuli. The key results of an investigation into auditory responses in the SHR revealed that the SC may also be hyper-responsive to stimuli, however, as there is a lower input (LFP response) into the SC in the SHR for auditory stimuli, it suggests a change in function upstream in these animals. The processing in the SC compensated for this reduced input, producing normalised output responses, and behavioural responses. The SHR consistently showed great onset latency responses for both visual and auditory stimuli, and is arguably due to a developmental delay in these animals.

It was hypothesised that if these differences in behaviour and physiology in the colliculus do indeed underlie symptoms of ADHD, then they should be normalised by treatments successfully prescribed for ADHD, including amphetamine. Therefore, an investigation was carried out into the effects of amphetamine on collicular visual and auditory responses in the SHR in comparison to WKY and WIS. In addition, the Hooded Lister rat (HL) was also included as an extra control strain because it has been used previously in studies investigating the effects of amphetamine. The main findings of the study were amphetamine caused a suppressive effect on visual responses, with a suppressive effect on auditory LFP responses only. The SHR were the least responsive to the drug, and did not normalise responses to comparable control levels. This suggests that amphetamine does have a locus

of action within the superficial layers of the SC, potentially reducing the distractibility in the SHR as well as in 'normal' control rats via a similar mechanism, despite not reducing the responses to normal levels. There was a lack of effect on auditory responses, perhaps due to a lack of monoamine innervation in these deeper layers in comparison to the superficial visual layers.

5.1. INTRODUCTION

Pharmacotherapies of ADHD such as amphetamines (D- and L-amphetamine sulphate isomers are used in the formulation of Adderall XR ®, Easton et al., 2007) have been found to be effective in reducing distractibility in ADHD (Brown and Cooke, 1994 and Spencer et al., 2001) and in healthy participants (Halliday et al., 1990 and Agmo et al., 1997). Also amphetamine improves sustained attention, and suppresses distractibility in rats (Evenden and Robbins, 1985; Agmo et al., 1997; Grilly, 2000; Bizarro et al., 2004). There have been mixed findings on the effects of amphetamine in 'normal' rats, such that amphetamine has been shown to increase (Bizarro et al., 2004) and decrease (Broos et al., 2012) response accuracy in a five-choice serial reaction time task, a test of attention and impulsivity. Animals that have a poor performance at baseline are more sensitive to the therapeutic effects of psychostimulants, showing improved attention, and a decrease in impulsivity (Blondeau and Dellu-Hagedorn, 2007; Puumala et al., 1996; Robinson, 2012). Importantly, the behavioural and cognitive deficits in the SHR seem to be responsive to the stimulants D- and L-amphetamine. It has been reported that the hyperactivity was ameliorated by treatment of D-amphetamine in SHR (Myers et al., 1982; Sagvolden et al., 1992). Also, Sagvolden and Xu (2008) tested ADHD-like behaviour and found D-amphetamine normalised SHR hyperactivity, impulsiveness and sustained attention, suggesting the SHR have predictive validity for amphetamine.

As previously discussed, the therapeutic mechanism of action of these drugs (and the underlying pathology) in ADHD has currently not fully understood (Spencer et al., 2002), as is also the case for their action on sustained attention in individuals without ADHD. The basic actions of amphetamine are to increase synaptic availability of the monoamines dopamine, noradrenalin (Azzaro and Rutledge, 1973; Easton et al., 2007) and, at higher doses, serotonin (Holmes and Rutledge, 1976; Kuczenski and Segal, 1989). However, exactly how these effects mediate the therapeutic action has not been identified. One possibility is that the drugs affect the SC, a structure that it is strongly implicated in behaviours that are core symptoms of ADHD (see Section 1.2.5), such as distractibility (Gaymard et al., 2003) and deficits in attention (Thorley, 1984). Recall from Section 1.2.6 that it is suggested that D-amphetamine amplifies the signal-to-noise ratio as it suppresses responses to stimuli which give relatively minimal levels of SC activation, as in the sub-optimal whole field light stimuli (Gowan et al., 2008), and augments responses to stimuli which give relatively high levels of activation, such as stimuli limited to the excitatory centre (Grasse et al., 1993). By efficiently decreasing the response, or 'bid' to weak stimuli, psychostimulants have the ability to bias the system so that 'bids' only arise in response to salient stimuli. This would therefore cause a reduction in overall distractibility and a correlative enhancement in sustained attention, as seen in normal people, and ADHD sufferers following psychostimulant administration.

In conclusion, there is evidence to suggest that the SC could be dysfunctional in ADHD and the SHR and that the therapeutic effect of psychostimulants in ADHD to decrease distractibility and improve sustained attention may be mediated by actions on the SC. The aim of the present study is to establish the effects of amphetamine on visual and auditory collicular responsiveness in the SHR. In particular, to determine whether amphetamine normalises the SHR strain differences seen in the visual and auditory responses in Chapter 3 and 4.

Hypotheses

- There will be a significant effect of amphetamine on visual and auditory responses in the SC in a manner that normalises responses in the SHR, with reference to the WKY, WIS and HL.

5.2. METHODS

A total of 108 rats were used for the experiments described (27 SHR; 26 WIS; 28 WKY; 27 HL). The weight of the animals immediately prior to experimentation is detailed, by strain and experiment, in Table 5.1. The normality of the weight data was confirmed using the Kolmogorov–Smirnov test and a One-Way ANOVA was conducted to examine where there were any strain differences in weight for each type of experiment. This revealed a significant difference in weight between the strains in the visual electrophysiological experiment ($F=8.62$; $df=3$; $p=0.0005$). Post hoc (Tukey HSD) analysis revealed the WIS had a significantly greater weight than the WKY ($p=0.001$), SHR ($p=0.0005$) and HL ($p=0.021$), there was no significant difference between any of the other three strains (WKY/SHR: $p=0.933$, WKY/HL: $p=0.720$, HL/SHR: $p=0.383$). Similar findings were seen in the auditory electrophysiological experiment ($F=7.03$; $df=3$; $p=0.0005$). Post hoc (Tukey HSD) analysis revealed the WIS had a significantly greater weight than the WKY ($p=0.0005$), SHR ($p=0.0005$) and HL ($p=0.0005$); there was no significant difference between any of the other three strains (WKY/SHR: $p=0.968$, WKY/HL: $p=0.903$, HL/SHR: $p=0.666$).

Experiment		SHR	WIS	WKY	HL
Vision Drug Electrophysiology	Number of subjects	11	11	12	11
	Mean weight ± SEM (g)	395.19±8.16	504.80±26.54	408.95±13.36	433.35±41.52
Auditory Drug Electrophysiology	Number of subjects	12	11	12	12
	Mean weight ± SEM (g)	405.05±6.73	520.15±17.34	413.19±12.56	425.33±12.72
Saline Electrophysiology	Number of subjects	4	4	4	4
	Mean weight ± SEM (g)	411.95±5.15	474.08±51.91	431.00±28.14	435.05±17.64

Table 5.1: The mean ±SEM weights (g) and number of subjects for the experiments within this chapter.

In the previous chapter, we examined differences in sensory responsiveness of the SHR, WIS and WKY and found some baseline differences. However, in this investigation Hooded Lister rats (HL) were also introduced due to their use in previous studies with amphetamine. A small number of animals received saline injections as a control measure and therefore an analysis of this condition was conducted for all four strains. This was followed by an analysis to determine the effects of amphetamine on each parameter (onset latency, amplitude, duration) using repeated measures ANOVA conducted with STRAIN as the between-subjects factor and DOSE as the within-subjects factor. All saline and drug experiments were carried out using the mid stimulus of the stimulus response curve.

The amphetamine doses used in the present chapter are 0.5, 1, 2, 4, 8 mg/Kg cumulatively. Schiffer et al. (2006) used PET to show that an amphetamine dose of 0.5 mg/Kg, i.v produced DAT occupancy levels in the primate brain equivalent to those achieved by the therapeutic human doses. This suggests that the lower i.v. administered doses of amphetamine used in the present study are arguably comparable to human relevant doses, but that our higher doses may have exceeded average therapeutic levels. Yet studies have been shown to use concentrations comparable to this study in rats to produce a behavioural effect to amphetamine (Gowan et al., 2008, Clements et al., 2014).

5.3. RESULTS

Inclusion criteria

The positions of the 45 visual recordings (11 SHR; 11 WIS; 12 WKY; 11 HL) used in the data analysis were all in the superficial layers of the SC, as shown in the reconstruction of the sections in Figure 5.1 and tabulated in Table 5.2 and Table 5.3. Of the 45 visual recordings used to compare amphetamine effects, 14 were positioned in the Opticum (Op; 3 SHR; 4 WIS; 3 WKY; 4 HL), 30 were recorded from the Superficial Grey (SuG; 8 SHR; 7 WIS; 8 WKY; 7 HL) and 1 was recorded in the Zonal Layer (Zo; 1 WKY). Of these recordings, 32 were

positioned in the medial SC (7 SHR; 8 WIS; 8 WKY; 9 HL), and 13 were positioned in the lateral SC (4 SHR; 3 WIS; 4 WKY; 2 HL). Of the 16 visual recordings used to compare saline effects (4 per strain), 7 were positioned in the Superficial Grey (2 SHR; 1 WIS; 2 WKY; 2 HL), and 9 were positioned in the Opticum (2 SHR; 3 WIS; 2 WKY; 2 HL). Of these saline recordings, 9 were positioned in the medial SC (3 SHR; 1 WIS; 2 WKY; 3 HL), and 7 were positioned in the lateral SC (1 SHR; 3 WIS; 2 WKY; 1 HL). Chi-square analysis showed there was no significant association between STRAIN and the positioning of the electrodes in terms of anterior-posterior positioning (χ^2=1.51; df=6; p=0.959), medial-lateral positioning (χ^2=1.04; df=3; p=0.791) or superficial layer positioning (χ^2=3.23; df=6; p=0.779).

Co-ordinates From Bregma	Layer	SHR N=11	WIS N=11	WKY N=12	HL N=11
-5.8mm	Zonal Layer	0	0	0	0
	Superficial Grey	1	1	1	1
	Opticum	0	1	0	0
-6.3mm	Zonal Layer	0	0	1	0
	Superficial Grey	5	4	6	4
	Opticum	2	3	1	2
-6.8mm	Zonal Layer	0	0	0	0
	Superficial Grey	2	2	1	2
	Opticum	1	0	2	2

Table 5.2: The anterior-posterior and layer positioning of the electrodes for the visual responses within the superficial layers of the superior colliculus for each strain. Chi-square analysis revealed no significant association.

	SHR	WIS	WKY	HL
Medial Recordings	7	8	8	9
Lateral Recordings	4	3	4	2

Table 5.3: The medial-lateral positioning of the electrodes for the visual responses. Chi-square analysis revealed no significant association.

The positions of the 46 auditory recordings (12 SHR; 11 WIS; 12 WKY; 11 HL) used in the data analysis were all in the deeper layers of the SC as shown in the reconstruction of the sections in Figure 5.3 and tabulated in Table 5.4. Of the 46 auditory responses used for stimulus response analysis, 23 were positioned in the Intermediate Grey (InG; 8 SHR; 5 WIS; 5 WKY; 5 HL), 2 were recorded from the Intermediate White (InW; 1 SHR; 1 HL), with the remaining 21 responses were recorded in the Deep Grey (DpG; 3 SHR; 6 WIS; 7 WKY; 5 HL). Of these recordings, 29 were positioned in the medial SC (7 SHR; 7 WIS; 7 WKY; 8 HL), and 17 were positioned in the lateral SC (5 SHR; 4 WIS; 5 WKY; 3 HL). Of the 16 auditory

recordings used to compare saline effects (4 per strain), 8 were positioned in the Intermediate Grey (InG; 2 SHR;, 1 WIS; 2 WKY; 3 HL), and 8 were positioned in the Deep Grey (2 SHR; 3 WIS; 2 WKY; 1 HL). Of these saline recordings, 9 were positioned in the medial SC (2 SHR; 3 WIS; 3 WKY; 1 HL), and 7 were positioned in the lateral SC (2 SHR; 1 WIS; 1 WKY; 3 HL). Chi-square analysis showed there were no significant association between STRAIN and the positioning of the electrodes in regarding anterior-posterior positioning (χ^2=1.56; df=6; p=0.955) medial-lateral positioning (χ^2=0.67; df=3; p=0.880), or deeper layer positioning (χ^2=4.67; df=6; p=0.587).

Co-ordinates From Bregma	Layer	SHR N=12	WIS N=11	WKY N=12	HL N=11
-5.8mm	Intermediate Grey	1	0	1	1
	Intermediate White	0	0	0	0
	Deep Grey	1	2	0	0
	Deep White	0	0	0	0
-6.3mm	Intermediate Grey	4	4	2	3
	Intermediate White	1	0	0	1
	Deep Grey	2	3	6	2
	Deep White	0	0	0	0
-6.8mm	Intermediate Grey	3	1	2	1
	Intermediate White	0	0	0	0
	Deep Grey	0	1	1	3
	Deep White	0	0	0	0

Table 5.4: The anterior-posterior and layer positioning of the electrodes for the auditory responses within the deeper layers of the superior colliculus for each strain. Chi-square analysis revealed no significant association.

	SHR	WIS	WKY	HL
Medial Recordings	7	7	7	8
Lateral Recordings	5	4	5	3

Table 5.5: The medial-lateral positioning of the electrodes for the auditory responses. Chi-square analysis revealed no significant association.

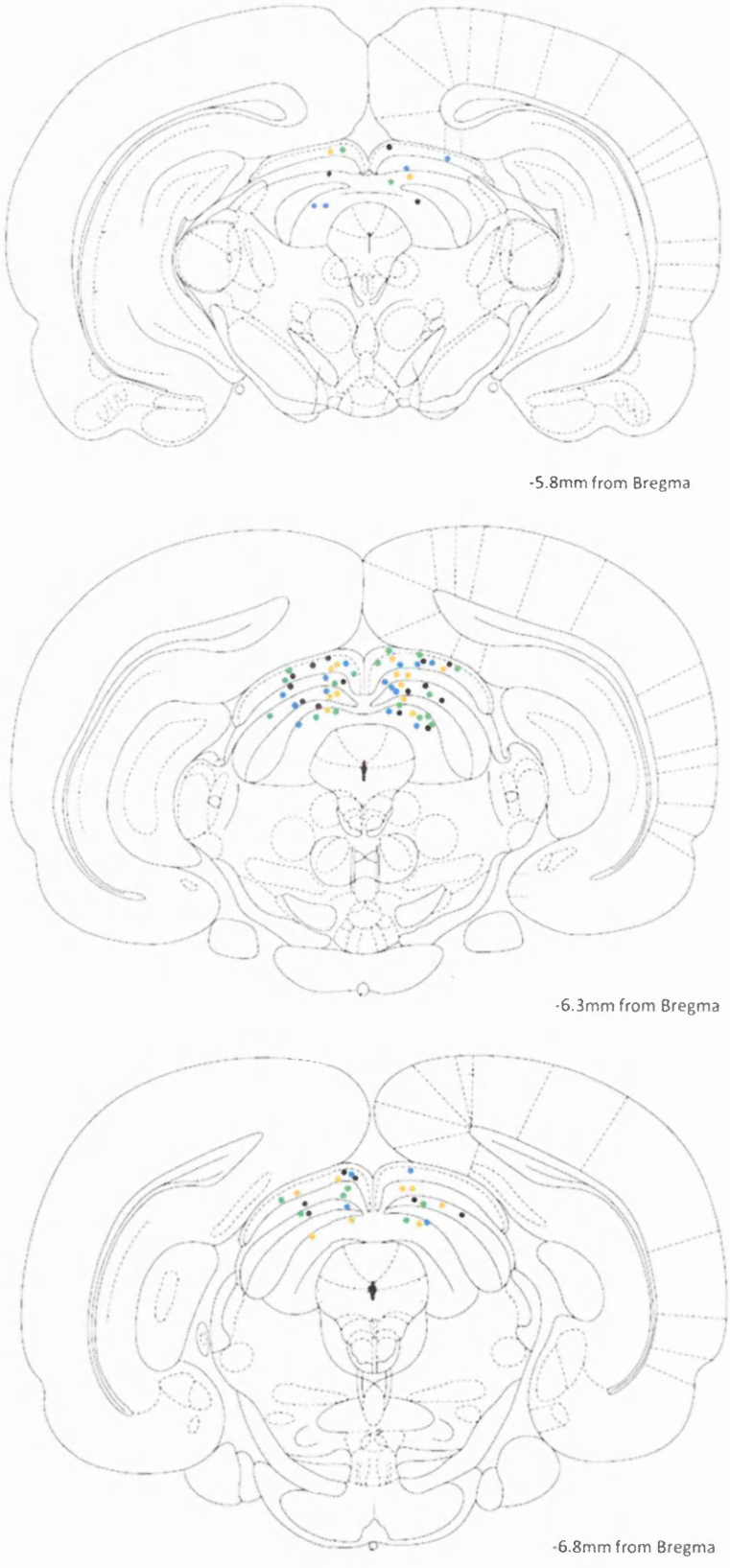

Figure 5.1: Reconstructed plots of recording sites in the SC. During collicular recordings, SHR recording sites are shown in black, WKY recording sites are shown in green, Wistar recording sites are shown in blue and HL recording sites are shown in yellow. The recording sites in the superficial layers are visual recordings, and the recording sites in the deeper layers are auditory responses. Adapted from Paxinos and Watson (1998).

Saline effects on visual and auditory responses

Equivalent volume cumulative doses of saline were administered as described in Section 2.3.3. Repeated measures ANOVA was conducted with STRAIN as the between-subjects factor and SALINE DOSE as the within-subjects factor (see Table 5.6). There was no main effect of dose on any parameter. There were also no significant interaction effects. For the majority of parameters there was no main effect of strain. However, there was a main effect of strain for multiunit activity auditory response duration. Post hoc (Tukey HSD) analysis found the HL had a significantly greater duration than the WIS strain only (p=0.037), while there were no other significant strain differences.

Experiment	Parameter	Main Effect of DOSE	Interaction	Main effect of STRAIN
LFP vision response	**Onset Latency**	F=1.31; df=3.00,0.10; p=0.285	F=1.33; df=8.99,0.25; p=0.259	F=1.56; df=3, 0.28; p=0.251
	Amplitude	F=0.30; df=1.24, 0.02; p=0.644	F=1.22; df=3.73, 0.23; p=0.343	F=0.86; df=3, 0.18; p=0.486
	Duration	F=2.44; df=1.50, 0.17; p=0.126	F=2.54; df=4.49, 0.39; p=0.071	F=0.58; df=3, 0.13; p=0.642
Multiunit activity vision response	**Onset Latency**	F=1.54; df=5, 0.11; p=0.191	F=0.77; df=15, 0.16; p=0.707	F=0.19; df=3, 0.04; p=0.904
	Amplitude	F=0.76; df=2.05, 0.06; p=0.481	F=0.98; df=6.14, 0.20; p=0.463	F=0.06; df=3, 0.01; p=0.982
	Duration	F=1.02; df=2.92, 0.08; p=0.395	F=0.67; df=8.76, 0.14; p=0.725	F=0.33; df=3, 0.08; p=0.806
LFP auditory response	**Onset Latency**	F=2.52; df=2.55, 0.16; p=0.111	F=1.03; df=7.65, 0.21; p=0.433	F=1.20; df=3, 0.23; p=0.352
	Amplitude	F=1.46; df=1.98, 0.11; p=0.252	F=1.30; df=5.93, 0.25; p=0.296	F=0.81; df=3, 0.17; p=0.513
	Duration	F=2.30; df=2.16, 0.16; p=0.117	F=0.92; df=6.47, 0.19; p=0.505	F=3.20; df=3, 0.45; p=0.062
Multiunit activity auditory response	**Onset Latency**	F=1.18; df=2.37, 0.09; p=0.329	F=0.97; df=7.11, 0.20; p=0.472	F=0.92; df=3, 0.19; p=0.463
	Amplitude	F=2.53; df=2.39, 0.17; p=0.089	F=1.14; 7.17, 0.22; p=0.367	F=1.65; df=3, 0.29; p=0.230
	Duration	F=1.08; df=5, 0.08; p=0.379	F=1.37; df=15, 0.26; p=0.193	F=4.20; df=3, 0.51; p=0.030

Table 5.6: The statistical analysis of the saline effects at each parameter.

5.3.1. LOCAL FIELD POTENTIAL VISUAL RESPONSE EFFECTS

As previously mentioned in Section 2.3.5, the vision data was filtered into LFP and multiunit activity. Visual local field potentials are considered as the synchronised input into the recording space, in this case the superficial layers of the superior colliculus. As high frequencies are filtered out, slower frequencies representing the postsynaptic potential, (i.e. excitatory postsynaptic potentials and inhibitory postsynaptic potentials) were kept for analysis.

Onset latency

A strain comparison of the baseline response was done using a one-way ANOVA to check for baseline differences because the HL rats were included in these experiments. There was no difference between the four strains for this parameter ($F=2.27$; $df=3$; $p=0.095$) prior to drug administration.

Amphetamine effects on visual LFP onset latency for each of the four strains are shown in Figure 5.2. There was no main effect of DOSE ($F=1.64$; $df=2.83, 0.04$; $p=0.186$). There was a significant main effect of STRAIN ($F=3.75$; $df=3, 0.22$; $p=0.018$), where overall the SHR had a significantly greater onset latency than the HL ($p=0.017$) and a trend towards a significantly greater onset latency than the WKY ($p=0.051$). There was no significant STRAIN x DOSE interaction ($F=1.25$; $df=8.50, 0.08$; $p=0.273$). Because there were no strain differences at baseline, additional repeated measures ANOVAs on the individual strains were conducted to see how the individual strains changed in onset latency with drug dose to result in the main effect of strain. The SHR showed no significant main effect of DOSE ($F=1.00$; $df=1.78$, $p=0.425$) indicated that for this strain there was no change in onset latency. However, there was a significant main effect of DOSE for the WKY strain ($F=6.48$; $df=5$; $p=0.0005$) and a trend towards a significant main effect of DOSE for the HL ($F=2.39$; $df=5$; $p=0.051$). In both cases latency increased with increasing dose, which would bring the

strains closer together and therefore it is not clear which changes contributed to the main effect of strain seen only in the overall analysis.

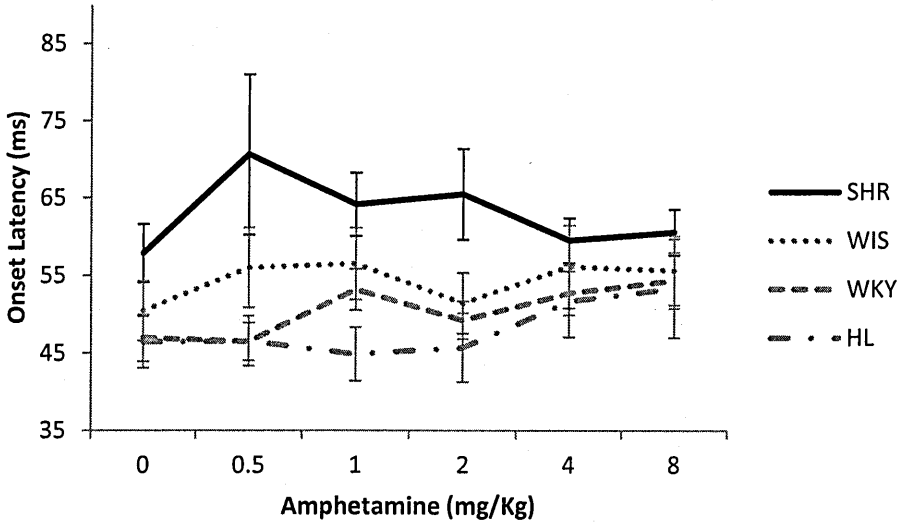

Figure 5.2: The mean ± SEM visual response LFP onset latency of the four strains over the increasing amphetamine doses, showing no main effect of DOSE. There was a main effect of STRAIN where the SHR had greater onset latency than the HL, and a trend towards a greater onset latency than the WKY. There was no DOSE x STRAIN interaction.

Amplitude

A strain comparison of the baseline response was done using a one-way ANOVA to check for baseline differences because the HL rats were included in these experiments. There was no difference between the four strains for this parameter (F=2.41; df=3; p=0.081) prior to drug administration.

Amphetamine effects on visual LFP amplitude for each of the four strains are shown in Figure 5.3. There was a main effect of DOSE (F=8.46; df=2.26, 0.17; p=0.0005). -subjects contrasts revealed that the lower dose of amphetamine (1 mg/Kg) caused a significant increase in amplitude (F=5.49; df=1, 0.12; p=0.024), yet the highest dose caused a significant decrease (F=6.56; df=1, 0.14; p=0.014), there were no significant differences of any other doses relative to baseline. There was no main effect of STRAIN (F=1.79; df=3,

0.12; p=0.165). There was no significant STRAIN x DOSE interaction (F=0.97; df=6.77, 0.07; p=0.459).

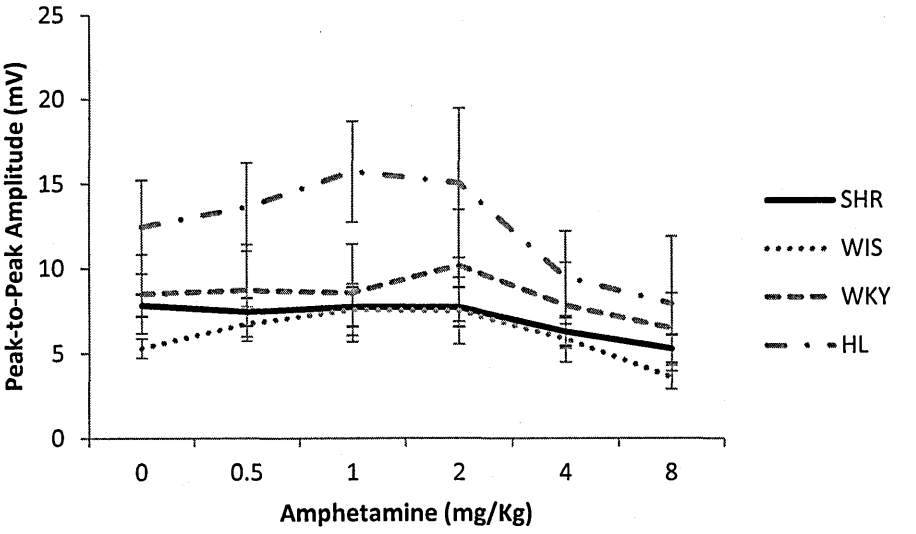

Figure 5.3: The mean ± SEM visual response LFP peak-to-peak amplitude of the four strains over the increasing amphetamine dose, showing a main effect of DOSE. The lower doses of amphetamine caused a significant increase in amplitude, yet the highest dose caused a significant decrease. There was no main effect of STRAIN or DOSE x STRAIN interaction.

Duration

A strain comparison of the baseline response was done using a one-way ANOVA to check for baseline differences because the HL rats were included in these experiments. There was a difference between the four strains for this parameter (F=16.84; df=3; p=0.0005) prior to drug administration. Post hoc (Tukey HSD) tests showed that the HL had a significantly larger duration than the SHR (p=0.0005), WIS (p=0.0005) and WKY (p=0.001). There were no other significant strain differences at baseline.

Amphetamine effects on visual LFP duration for each of the four strains are shown in Figure 5.4. There was a main effect of DOSE (F=6.85; df=3.14, 0.14; p=0.0005). Amphetamine caused a significant decrease in response duration at dose 4mg/Kg (F=18.34; df=1, 0.31;

p=0.0005) and 8mg/Kg (F=8.21; df=1, 0.17; p=0.007), there were no significant differences of any other doses relative to baseline. There was a main effect of STRAIN (F=19.07; df=3, 0.58; p=0.0005). Post hoc (Tukey HSD) analysis showed the HL had a significantly longer response duration than the SHR (p=0.0005), WIS (p=0.0005) and WKY (p=0.0005). This mirrors the differences seen at baseline. There was no significant STRIAN x DOSE interaction (F=1.54; df=9.41, 0.10; p=0.138).

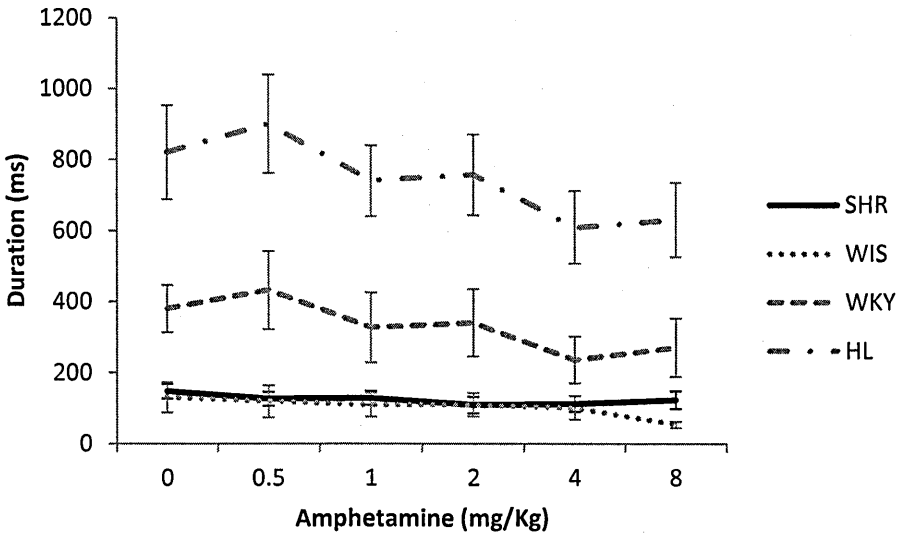

Figure 5.4: The mean ± SEM visual LFP duration of the four strains over the increasing amphetamine dose showing a significant main effect of dose, with a decreasing duration as dose increased. There was a main effect of strain, the HL had a significantly longer duration than the three albino strains, although this was also present at baseline. There was no interaction between strain and dose.

In summary, amphetamine caused a significant increase in LFP amplitude at the lowest dose (0.5 mg/Kg), but the higher doses caused a significant decrease in LFP amplitude (8 mg/Kg) and duration (4-8 mg/Kg). The SHR had greater onset latency than the HL, and a trend towards greater onset latency than the WKY. These differences were not seen at pre-drug baseline. There was no significant interaction for any parameter, suggesting all animals reacted to the drug similarly.

5.3.2. MULTIUNIT ACTIVITY VISUAL RESPONSE EFFECTS

As previously mentioned in Section 2.3.5, the vision data was filtered into LFP and multiunit activity. The visual multiunit activity data represents the output from the area, in this case the superficial layers of the superior colliculus. The fast frequencies are mostly caused by the short inward and outward currents of action potentials, representing the spike activity of neurons and were kept for analysis.

Onset latency

A strain comparison of the baseline response was done using a one-way ANOVA to check for baseline differences because the HL rats were included in these experiments.. There was no difference between the four strains for this parameter ($F=2.07$; $df=3$; $p=0.119$) prior to drug administration.

Amphetamine effects on visual multiunit activity onset latency for each of the four strains are shown in Figure 5.5. There was a main effect of DOSE ($F=5.37$; $df=2.66, 0.12$; $p=0.003$). Within-subjects contrasts revealed that amphetamine caused a significant increase in response onset latency at the highest dose, dose 8 mg/Kg only ($F=5.77$; $df=1, 0.12$; $p=0.021$), there were no significant differences of any other doses relative to baseline. There was no main effect of STRAIN ($F=2.17$; $df=3, 0.14$; $p=0.106$). There was no DOSE x STRAIN interaction ($F=0.64$; $df=7.97, 0.05$; $p=0.743$). In line with the results of Gowan et al. (2008), it is worth noting that there was no main effect of DOSE for the HL strain when this was considered in an independent analysis ($F=1.02$; $df=2.87$; $p=0.397$).

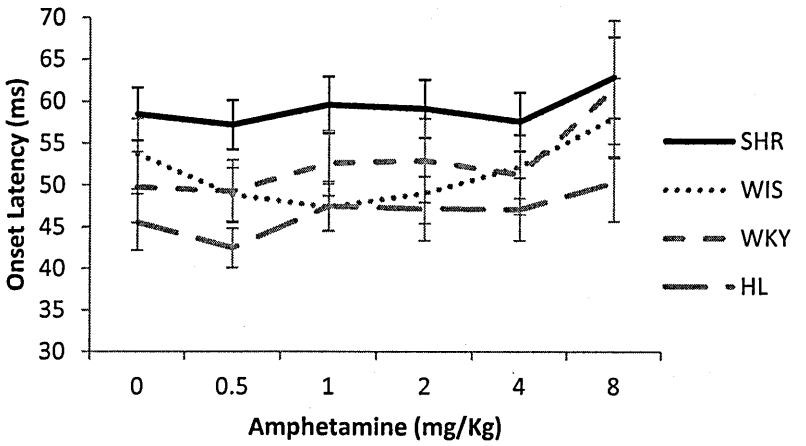

Figure 5.5: The mean ± SEM visual response onset latency of the four strains over the increasing amphetamine dose showing a significant main effect of dose, with increasing onset latency at the final dose. There were no main effects of strain or interactions between strain and dose.

Amplitude

A strain comparison of the baseline response was done using a one-way ANOVA to check for baseline differences because the HL rats were included in these experiments. There was a difference between the four strains for this parameter (F=6.49; df=3; p=0.001) prior to drug administration. Post hoc (Tukey HSD) tests showed that the SHR had a significantly larger amplitude than the WIS (p=0.012) and WKY (p=0.038); the HL also had a significantly larger amplitude than the WIS (p=0.008) and WKY (p=0.025). There were no significant differences between the WIS and WKY (p=0.953), and between the SHR and HL (p=0.998).

Amphetamine effects on visual multiunit activity amplitude for each of the four strains are shown in Figure 5.6. There was a main effect of DOSE (F=14.30; df=2.42, 0.26; p=0.0005). The highest two doses; 4mg/Kg (F=4.81; df=1, 0.11; p=0.034) and 8 mg/Kg (F=37.35; df=1, 0.48; p=0.0005) caused a significant decrease in response amplitude, there were no significant differences of any other doses relative to baseline.

There was no main effect of STRAIN (F=2.62; df=3, 0.16; p=0.064). Given there were strain differences at baseline, additional repeated measures ANOVAs on the individual strains were conducted to see how the individual strains changed in response amplitude from baseline with drug dose to result in the main effect of strain being lost from baseline. Analysis of the SHR showed a main effect of DOSE (F=4.32; df=18.24, 1.82; p=0.032). Within-subjects contrasts revealed that there was a significant decrease in amplitude for this strain from baseline only at the highest dose (p=0.028). Analysis of WIS responses found a significant main effect of DOSE (F=3.50; df=21.09, 2.11; p=0.047) but within-subjects contrasts did not find any significant difference from baseline for any dose Analysis of the WKY responses also revealed a significant main effect of DOSE (F=3.72; df=11, 1 p=0.006) but no doses actually differing from baseline. This suggests that changes to the WIS and WKY responses did not contribute to the loss of the significant difference at baseline. Finally, analysis of the HL responses also showed a main effect of DOSE (F=6.75; df=19.94, 1.81; p=0.007) with the 4 mg/Kg (p=0.049) and 8 mg/Kg (p<0.001) doses producing significant decreases in amplitude. In sum, these analyses indicate that the loss of the initial baseline differences likely rose from changes to both the SHR and HL response amplitudes at the higher doses.

Returning to the main analysis, this is supported by a significant DOSE x STRAIN interaction (F=2.16; df=7.25, 0.14; p=0.042). There was a trend towards a significant interaction between the SHR and HL (F=3.13; df=1.93, 0.14; p=0.057), similarly there was a significant interaction between these two strains between the baseline and final dose (F=8.58; df=1, 0.30; p=0.008), yet there were no significant interactions between the dose compared to the following cumulative dose between these strains. Overall, the HL showed a greater dose-dependent decrease in amplitude than the SHR.

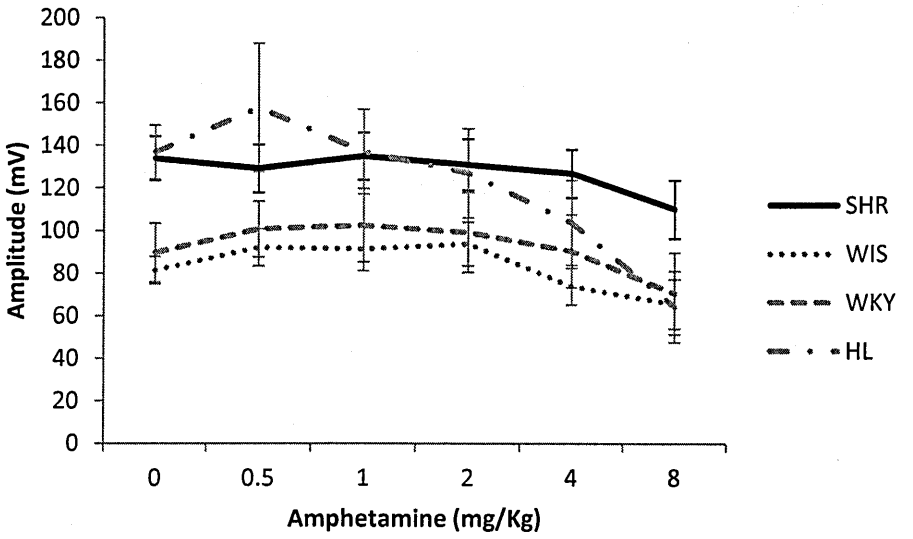

Figure 5.6: The mean ± SEM visual response multiunit activity amplitude of the four strains over the increasing amphetamine dose showing a significant main effect of dose, with a decreasing amplitude at the highest two doses; there were no main effects of strain. There was an interaction between strain and dose. Overall, the HL showed a significantly greater decline in amplitude than the SHR.

Duration

A strain comparison of the baseline response was done using a one-way ANOVA to check for baseline differences because the HL rats were included in these experiments. There was a difference between the four strains for this parameter (F=7.10; df=3; p=0.001) prior to drug administration. Post hoc (Tukey HSD) tests showed that the HL had a significantly larger duration than the WIS (p=0.001) and SHR (p=0.004). There were no significant differences between the HL and WKY (p=0.204). There were no other significant strain differences at baseline.

Amphetamine effects on visual multiunit activity duration for each of the four strains are shown in Figure 5.7. There was a main effect of DOSE (F=16.12; df=2.83, 0.28; p=0.0005). Amphetamine caused a significant decrease in response duration at dose 4mg/Kg (F=12.59; df=1, 0.24; p=0.001) and 8mg/Kg (F=26.88; df=1, 0.40; p=0.0005), there were no significant differences of any other doses relative to baseline. There was a main effect of STRAIN

(F=7.40; df=3, 0.35; p=0.0005). Post hoc (Tukey HSD) analysis showed the HL had a significantly longer response duration than the SHR (p=0.004), WIS (p=0.0005) and WKY (p=0.035). There were no strain differences between the albino strains. This is similar to the significant differences found at baseline where the HL had a significantly longer duration than the WIS and SHR. In order to establish whether the new difference seen here between HL and WKY was due to one or both strains changing, additional repeated measures ANOVAs were done for each strain individually. The WKY responses showed a main effect of DOSE (F=8.55; df=18.42, 1.67; p=0.003). Within-subjects contrasts revealed that the 2 mg/Kg (p=0.031) 4 mg/Kg (p=0.012) and 8 mg/Kg (p=0.007) resulted in a significant decrease from baseline. Analysis of HL data also revealed a significant main effect of DOSE (F=13.01; df=21.65, 1.97; p<0.001) with within-subjects contrasts also showing decreases in duration at 4 mg/Kg (p=0.008) and 8 mg/Kg (p=0.002) doses. This suggests that changes to both strains could have contributed to the appearance of a significant difference in duration that was not present at baseline.

Again returning to the main analysis, there was a DOSE x STRAIN interaction (F=2.15; df=8.49, 0.14; p=0.034). Restricted ANOVA revealed this was likely due to, in part, a significant interaction between the WIS and HL (F=3.40; df=2.47, 0.15; p=0.032). This was significant interaction between these two strains between the baseline and the 4 mg/Kg dose (F=4.76; df=1, 0.19; p=0.041) and 8mg/Kg dose (F=4.68; df=1, 0.19; p=0.043), yet there were no significant interactions between these doses compared to the following cumulative dose between these strains. At the final two doses, the HL showed a greater decline in response duration than the WIS. There was a trend towards a significant interaction between the WIS and WKY (F=2.67; df=2.93, 0.11; p=0.056), similarly there was a trend towards a significant interaction between these two strains between the baseline and the 2 mg/Kg (F=4.01; df=1, 0.16; p=0.058) and a significant interaction between the baseline and 4 mg/Kg dose (F=4.82; df=1, 0.19; p=0.040). There was a trend towards a

significant interaction between the 2 mg/Kg and 4 mg/Kg dose between these strains (F=3.92; df=1, 0.16; p=0.061). The WKY showed a greater decline in response duration than the WIS between these doses.

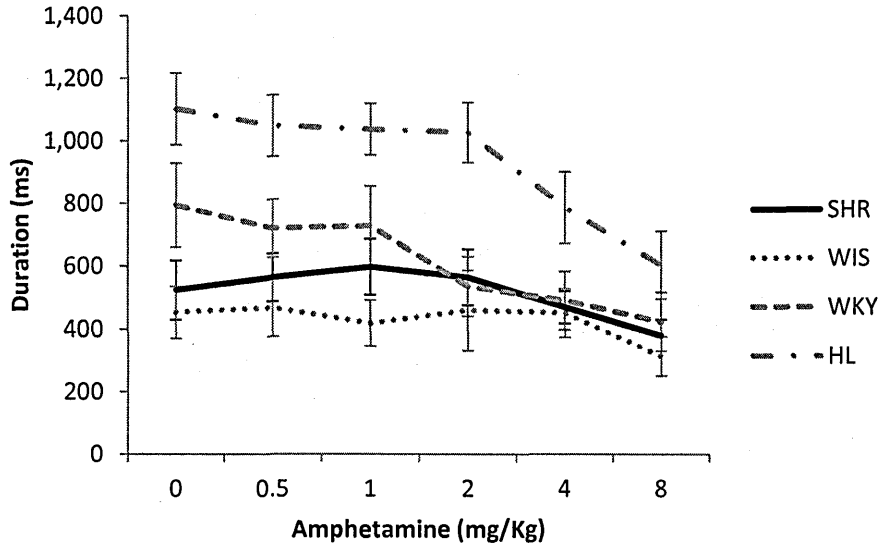

Figure 5.7: The mean ± SEM visual response multiunit activity duration of the four strains over the increasing amphetamine dose showing a significant main effect of dose, with a decreasing duration as dose increased. The HL had a significantly longer response duration than the all the albino strains. At the final two doses the HL showed a greater decline in response duration than the WIS. The WKY showed a greater decline in response duration than the WIS between the 2 mg/Kg and 4 mg/Kg doses.

In summary, higher doses (4-8 mg/kg) of amphetamine caused a significant increase in onset latency and a decrease in amplitude and duration. The SHR had a lesser dose-dependent decrease in multiunit activity amplitude than the HL, yet the decline in SHR amplitude did not reach normal WIS and WKY baseline levels. Similarly, at the final two doses, the HL showed a greater decline in response duration than the WIS. HL had significantly longer response duration than the albino strains. The WKY also showed a greater decline in response duration than the WIS between the 2 mg/Kg and 4 mg/Kg doses. It suggests that the HL was most responsive to the drug, in comparison to the albino animals, the WIS showed least response effect of drug on duration.

5.3.3. LOCAL FIELD POTENTIAL AUDITORY RESPONSE EFFECTS

As previously mentioned in Section 2.3.5, the data was filtered into LFP and multiunit activity. Auditory local field potentials are considered as the synchronised input into the recording space, in this case the deeper layers of the superior colliculus.

Onset latency

A strain comparison of the baseline response was done using a one-way ANOVA to check for baseline differences because the HL rats were included in these experiments. There was no difference between the four strains for this parameter (F=0.87; df=3; p=0.466) prior to drug administration.

Amphetamine effects on auditory LFP onset latency for each of the four strains are shown in Figure 5.8. There was no main effect of DOSE (F=1.32; df=3.88, 0.03; p=0.265). There was no main effect of STRAIN (F=1.89; df=3, 0.12; p=0.146). There was no DOSE x STRAIN interaction (F=1.07; df=11.63, 0.07; p=0.386).

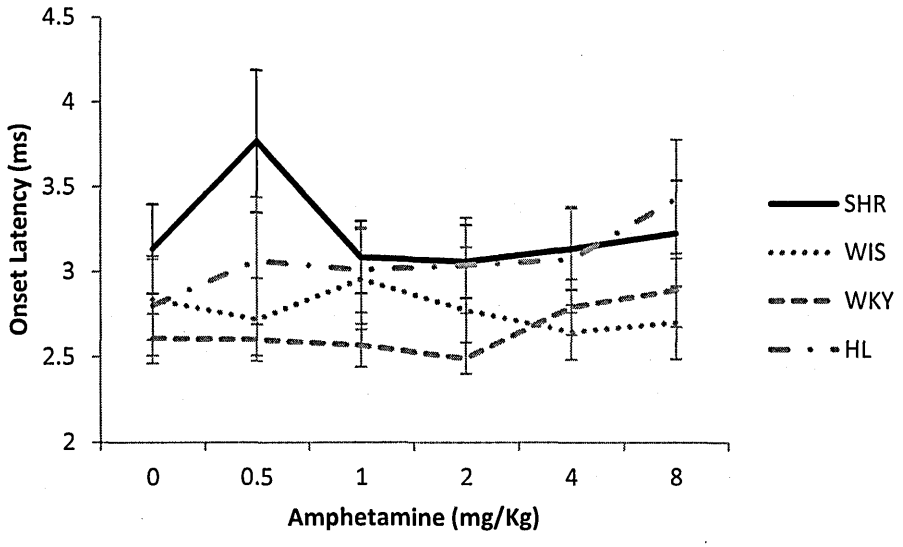

Figure 5.8: The mean ± SEM auditory response LFP onset latency of the four strains over the increasing amphetamine dose showing no significant main effect of dose, strain or interactions between strain and dose.

Amplitude

A strain comparison of the baseline response was done using a one-way ANOVA to check for baseline differences because the HL rats were included in these experiments. There was a difference between the four strains for this parameter (F=5.79; df=3; p=0.002) prior to drug administration. Post-hoc (Tukey HSD) tests showed that the SHR had a significantly smaller amplitude than the WKY (p=0.007) and HL (p=0.009). There were no other significant strain differences seen at baseline.

Amphetamine effects on auditory LFP amplitude for each of the four strains are shown in Figure 5.9. There was a main effect of DOSE (F=3.61; df=3.28, 0.08; p=0.012), with doses 1mg/Kg (F=7.35; df=1, 0.15; p=0.010); 2mg/Kg (F=7.35; df=1, 0.11; p=0.031); 4mg/Kg (F=4.03; df=1, 0.09; p=0.051) and 8mg/Kg (F=9.57; df=1, 0.19; p=0.004) causing a significant decrease in response amplitude relative to baseline, there were no significant differences of any other doses relative to baseline. There was a main effect of STRAIN (F=6.64; df=3, 0.32; p=0.001). Post hoc (Tukey HSD) analysis showed the SHR strain having a significantly lower response amplitude that the WKY (p=0.004) and HL (p=0.003) as expected from baseline. There was no DOSE x STRAIN interaction (F=1.03; df=9.84, 0.07; p=0.426) for this parameter.

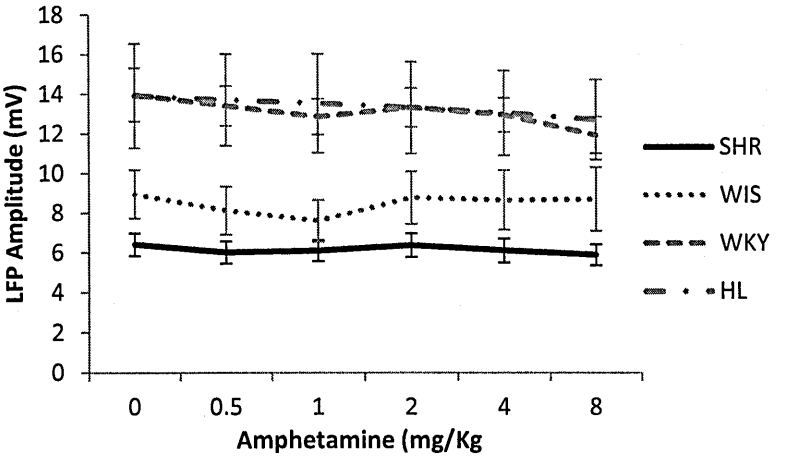

Figure 5.9: The mean ± SEM SEM auditory response LFP peak-to-peak amplitude of the four strains over the increasing amphetamine dose showing a significant main effect of dose, the final four doses causing a significant decrease in amplitude. The SHR had significantly lower response amplitude than the WKY and HL but this was also found at baseline. There was no interaction between strain and dose.

Duration

A strain comparison of the baseline response was done using a one-way ANOVA to check for baseline differences because the HL rats were included in these experiments. There was no difference between the four strains for this parameter (F=2.12; df=3; p=0.112) prior to drug administration.

Amphetamine effects on auditory LFP duration for each of the four strains are shown in Figure 5.10. There was no main effect of DOSE (F=1.04; df=3.25, 0.02; p=0.379). There was a main effect of STRAIN (F=4.18; df=3, 0.23; p=0.011). Post hoc (Tukey HSD) analysis showed the HL strain had significantly longer response duration than the WKY (p=0.042) and SHR (p=0.010). Given there were no strain differences at baseline, additional repeated measures ANOVAs on the individual strains were conducted to see how the individual strains changed in response duration from baseline with drug dose to result in the main effect of strain being found. Analysis of HL responses showed no significant main effect of DOSE (F=1.76; df=50. 5; p=0.138). There was also no main effect of DOSE when the WKY

were analysed alone (F=0.68; df=16.70, 1.52; p=0.482) or the SHR (F=0.25; df=16.62, 1.51; p=0.718). This suggests that any changes arising to cause the main effect of DOSE in the main analysis that were not due to changes in any one strain. This is supported by a lack of significant DOSE x STRAIN interaction (F=1.34; df=9.74, 0.09; p=0.219) in the main analysis.

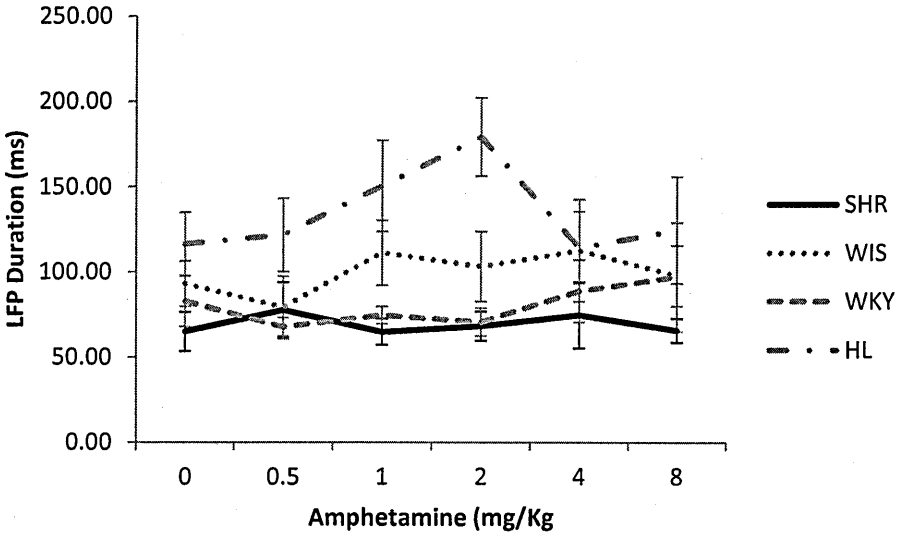

Figure 5.10: The mean ± SEM auditory response LFP duration of the four strains over the increasing amphetamine dose showing no significant main effect of dose. The HL had significantly longer response duration than the WKY and SHR. There was no interaction between strain and dose.

In summary, amphetamine caused a significant dose-dependent decrease in amplitude. The SHR had lower response amplitude than the HL and WKY, a difference also seen at baseline. The HL had a significantly longer duration than the WKY and SHR. There was no significant interaction for any parameter, therefore all animals responded to the drug similarly.

5.3.4. MULTIUNIT ACTIVTY AUDITORY RESPONSE EFFECTS

As previously mentioned in Section 2.3.5, the vision data was filtered into LFP and multiunit activity. The auditory multiunit activity data represents the output from the area, in this case the deeper layers of the superior colliculus.

Onset latency

A strain comparison of the baseline response was done using a one-way ANOVA to check for baseline differences because the HL rats were included in these experiments. There was no difference between the four strains for this parameter (F=2.41; df=3; p=0.081) prior to drug administration.

Amphetamine effects on auditory multiunit activity onset latency for each of the four strains are shown in Figure 5.11. There was no main effect of DOSE (F=1.74; df=3.29, 0.04; 0.157). There was no main effect of STRAIN (F=2.29; df=3, 0.14; p=0.092). There was no DOSE x STRAIN interaction (F=0.72; df=9.86, 0.05; p=0.705).

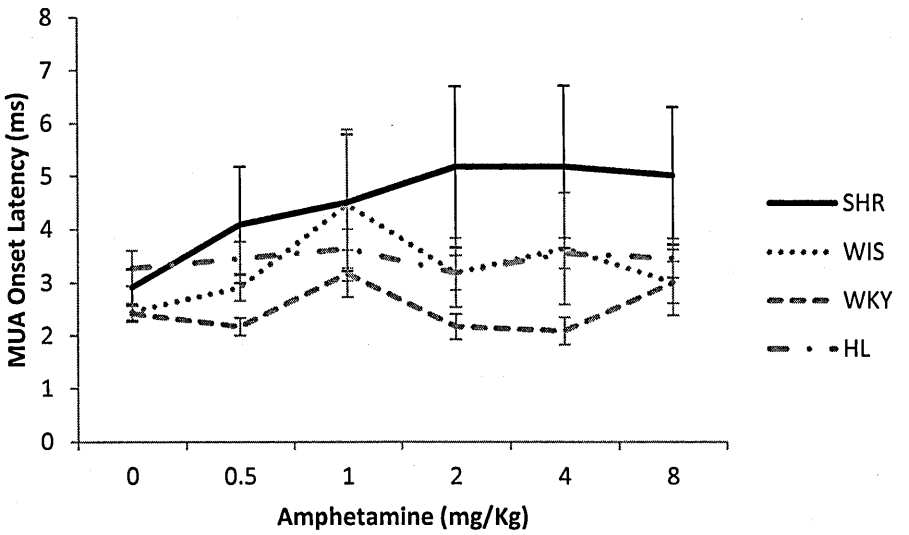

Figure 5.11: The mean ± SEM multiunit auditory response onset latency of the four strains over the increasing amphetamine dose showing no significant main effect of dose, strain or interactions between strain and dose.

Amplitude

A strain comparison of the baseline response was done using a one-way ANOVA to check for baseline differences because the HL rats were included in these experiments. There was a difference between the four strains for this parameter (F=11.14; df=3; p=0.0005) prior to

drug administration. Post-hoc (Tukey HSD) showed that the WKY had a significantly larger amplitude than the WIS (p=0.037), SHR (p=0.0005) and HL (p=0.002). The SHR had a significantly smaller amplitude than the WIS (p=0.048).

Amphetamine effects on auditory multiunit activity onset latency for each of the four strains are shown in Figure 5.12. There was no main effect of DOSE (F=0.63; df=2.57, 0.02; p=0.573). There was a main effect of STRAIN (F=9.60; df=3, 0.41; p=0.0005). Post hoc (Tukey HSD) revealed the WKY strain had significantly higher response amplitude than the SHR (p=0.0005) and HL (p=0.008) but not the WIS (p=0.063). This is slightly different from baseline where the WKY was significantly larger than the WIS and the WIS was significantly larger than the SHR. In order to evaluate what changes may have contributed to this change in relationship between the WKY, WIS and SHR, individual strain data was analysed using a repeated measures ANOVA. There was no main effect of DOSE when SHR (F=0.58; df=55, 5; p=0.714), WKY (F=2.11; df=55, 5; p=0.078) or WIS (F=0.48; df=14.09, 1.41; p=0.566) data was considered indicating that this slight change in strain differences beyond baseline was not due to changes in any one strain. This is supported by a lack of significant DOSE x STRAIN interaction (F=1.54; df=7.70, 0.10; p=0.154) in the main analysis.

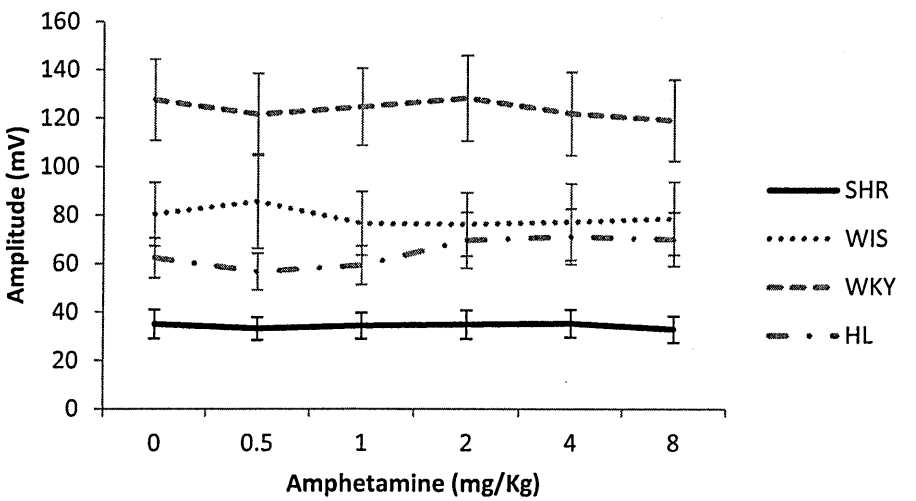

Figure 5.12: The mean ± SEM multiunit auditory response amplitude of the four strains over the increasing amphetamine dose showing no significant main effect of dose. The WKY had significantly higher response amplitude than the SHR and HL. There was no interaction between strain and dose.

Duration

A strain comparison of the baseline response was done using a one-way ANOVA to check for baseline differences because the HL rats were included in these experiments There was no difference between the four strains for this parameter (F=1.82; df=3; p=0.159) prior to drug administration.

Amphetamine effects on auditory multiunit activity duration for each of the four strains are shown in Figure 5.13. There was no main effect of DOSE (F=1.10; df=1.91, 0.03; p=0.337). There was no main effect of STRAIN (F= F=2.55; df=3, 0.15; p=0.068). There was no DOSE x STRAIN interaction (F=0.70; df=5.74, 0.05; p=0.648).

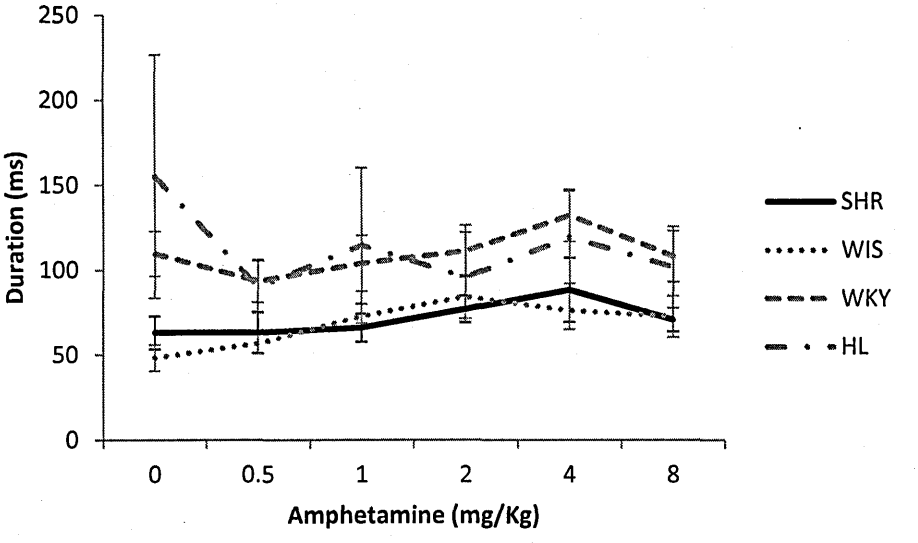

Figure 5.13: The mean ± SEM multiunit auditory response duration of the four strains over the increasing amphetamine dose showing no significant main effect of dose, strain or interactions between strain and dose.

In summary, amphetamine had no significant main effect on any parameter, therefore SHR responses were not normalised to comparable control strain baseline responses. The SHR had significantly lower response amplitude than the WKY, the WKY also had significantly higher response amplitude than the HL, both differences were seen at baseline. There was no

significant interaction for any parameter, therefore all animals responded to the drug similarly.

5.4. DISCUSSION

In summary, amphetamine caused a significant increase in superficial layer LFP amplitude at the lowest dose, and at the highest dose it caused a significant decrease in LFP (decrease in amplitude and duration) and multiunit (increase in onset latency, decrease in amplitude and duration) visual responses. The SHR had a greater LFP onset latency than the HL, yet this was not seen at the pre-drug baseline. When the strains were analysed alone, the HL had a trend towards a significant increase in onset latency, and the WKY had a significant increase in onset latency as the doses increased. In both cases latency increased with increasing dose, which would bring the strains closer together and therefore it is not clear which changes contributed to the main effect of strain seen only in the overall analysis. There was no significant interaction for any LFP parameter; therefore all animals reacted to the drug similarly. The HL and SHR both had significantly higher multiunit response amplitudes at baseline than the albino control strains. Yet, the SHR had a lesser dose-dependent decrease in multiunit activity amplitude than the HL, and therefore unlike the HL, the decline in SHR amplitude did not reach normal WIS and WKY baseline levels.

Amphetamine caused a significant dose-dependent decrease in LFP amplitude to auditory stimuli, yet there were no effects of amphetamine on multiunit activity auditory responses. The SHR had lower LFP response amplitude than the HL and WKY, yet the WKY had significantly higher multiunit response amplitude than the SHR and HL. There was no significant interaction for any parameter, therefore all animals responded to the drug similarly.

The effects of amphetamine on visual responses are comparable to the findings of Gowan et al. (2008) who showed that the key effects of d-amphetamine were to decrease the amplitude and duration of visually evoked activity in the superficial layers of the SC as measured by both LFPs and multiunit activity. Yet, unlike the results of the Gowan et al. (2008), a dose-dependent increase in multiunit activity onset latency was found in this study, suggesting a possible difference in albino strains and pigmented strains, as it was found that amphetamine caused no effect on this parameter for the HL strain. Similarly this finding is only significant at the highest dose for the albino strains. Given that LFPs embodies (in large part) the sum of post-synaptic potentials (Mitzdorf, ,1987), these findings indicate that d-amphetamine depresses both the input to (synaptic activity) and output from (spike activity) the superficial layers of SC. Further evidence of the validity of this study and a comparable effect in humans is that Chee (1991) observed the incidence of spontaneous emissions of high-voltage alpha wave electrophysiological activity in the SC was suppressed by increasing doses of D-amphetamine. However, in the cat, D-amphetamine augmented responses in the superficial layers of the SC when a stimulus was displayed within the excitatory centre of the cells' receptive fields only (Grasse et al., 1993). It is possible that D-amphetamine amplifies the signal-to-noise ratio as it suppresses responses to stimuli which give relatively minimal levels of SC activation, as in the sub-optimal whole field light stimuli (Gowan et al., 2008), and augments responses to stimuli which give relatively high levels of activation, such as stimuli limited to the excitatory centre (Grasse et al., 1993).

The SC is strongly implicated in saccade generation as well as in complex tasks involving attention (see Section 1.2.5), could imply that the drug's locus of action is on the SC. There are many reports of saccade abnormalities in ADHD with people with ADHD having deficits in saccade inhibition (Armstrong and Munoz, 2003; Klein et al., 2003; Munoz et al., 2003; Feifel et al., 2004; O'Driscoll et al,. 2005). Interestingly, in a MGS task, Mostofsky et al.

(2001) found unmedicated children with ADHD showed longer saccade latency, while those medicated with the psychostimulant, methylphenidate, had a drastic reduction in saccade latency, such that it became comparable to that of the age-matched control group. The SHR had a greater LFP onset latency than the HL, yet this was not seen in the pre-drug baseline analysis. When the strains were analysed alone, the HL had a trend towards a significant increase in onset latency, and the WKY had a significant increase in onset latency as the doses increased. In both cases latency increased with increasing dose, which would bring the strains closer together and therefore it is not clear which changes contributed to the main effect of strain seen only in the overall analysis. Similarly, there were no differences in the multiunit activity unlike what was seen in Chapter 3. This suggests that a great number of subjects may be needed to show the difference in baseline results, or that the differences seen in Chapter 3 are more pronounced at lower light intensities and are due to a dysfunction in the signal-to-noise ratio that would not be a factor on a mid-stimulus of a stimulus curve.

As previously mentioned, the HL and SHR both had significantly higher multiunit response amplitudes at baseline than the albino control strains. Yet, the SHR had a lesser dose-dependent decrease in multiunit activity amplitude than the HL. Therefore unlike the HL, the decline in SHR amplitude did not reach normal WIS and WKY baseline levels. It suggests that the HL was most responsive to the drug, in comparison to the albino animals, with the SHR being least responsive. Even though the effects of amphetamine reduced the increased responsiveness in the SHR seen in Chapter 3, it did not normalise the response, and thus, presumably normalise behavioural differences, suggesting a lack of predictive validity of this strain. Yet, the results of the present study suggest that d-amphetamine may (at least in part) act on the SC, albeit it in a manner which affects those with and without ADHD similarly. Such action could provide a locus of action to alter distractibility and sustained attention in SHR, and 'normal' rats, as well as individuals with ADHD and healthy

individuals. This would be in line with previous research reporting similar effects of amphetamine improving sustained attention, and suppressing distractibility in individuals without ADHD (Mackworth, 1965; Silber et al., 2006; Halliday et al., 1990; Agmo et al., 1997), as well as in individuals with ADHD (Oades, 1987; Brown and Cooke, 1994; Spencer et al., 2001; Wigal et al., 2005; Faraone et al., 2007). Similar to the findings of pschostimulant effects on normal subjects, amphetamine has also been shown to improve sustained attention, and suppresses distractibility behaviours in rats (Bizarro et al., 2004; Evenden and Robbins, 1985; Agmo et al., 1997; Grilly, 2000; Bizarro et al., 2004), as well as to normalise ADHD-like behaviours seen in the SHR (Sagvolden and Xu, 2008).

The implications of amphetamines effects from a behavioural stand point is that it has been hypothesised that the SC could 'bid' for motor expression, thus, heightened activity can be thought of as placing a stronger "bid" into the central selection device thought to be the basal ganglia (Chevalier and Deniau, 1990). This would therefore increase the likelihood of saccade generation. The superficial layers of the SC have a direct ascending projection to the thalamus and then forward to the neostriatum (McHaffie et al., 2005), and also connections with the deep layers of the SC (Lee et al., 1997), which also project to the thalamus (McHaffie et al., 2005). By efficiently decreasing the response, or 'bid' to weak stimuli, psychostimulants have the ability to bias the system so that distractions (a motor output and hence a saccade) only arise to predominantly salient stimuli. By enhancing SC responses, the likelihood of a saccade could be improved. Conversely, as is the case with d-amphetamine, by depressing responses in the SC, the prospect of a saccade would be lowered. This would therefore cause a reduction in overall distractibility and a correlative enhancement in sustained attention, as seen in normal people, ADHD sufferers, as well as rats (and more specifically the SHR) following psychostimulant administration.

Amphetamine caused a significant dose-dependent decrease in LFP amplitude to auditory stimuli, yet there were no effects of amphetamine on multiunit activity auditory responses, suggest that amphetamine may of causing an effect upstream of the SC, yet not locally within the deeper layers of the SC. Similarly to the results of Chapter4, the SHR had lower LFP response amplitude than the HL and WKY, yet this was not normalised to control levels. D-amphetamine has been shown to act locally within the superficial layers of the SC to affect visual responsiveness, yet the lack of amphetamine effects on multiunit activity auditory responses shown in this study could be due to the distinct differences in the distribution of neurotransmitters within the SC. For example one of the most prominent collicular neurotransmitters, acetylcholine is reportedly homogeneously distributed within the superficial layers, but occurs in patches or clusters within the deeper layers (McHaffie et al., 1986; Wallace, 1986; Wiener, 1986). Similarly Gamma-aminobutyric acid (GABA), the second most prominent collicular neurotransmitter, is found within the deeper layers of the SC (Adachi et al., 2003), but is most abundant in the superficial layers (Mize and Horner, 1985; Mize, 1986). As previously mentioned, an increase in synaptic availability of the monoamines dopamine, noradrenalin (Azzaro and Rutledge, 1973; Easton et al., 2007) and at higher doses serotonin (Holmes and Rutledge, 1976; Kuczenski and Segal, 1989) mediates the acute effect of amphetamine administration. Expansive serotonergic innervation, preferentially innervating the superficial layers is found in the SC (Parent et al., 1981; Weller et al., 1987), while restricted noradrenergic (Lindvall and Bjorklund, 1974; Weller et al., 1987) and dopaminergic input (Weller et al., 1987; Campbell et al., 1991) have been reported. Similarly, the concentration of endogenous noradrenaline was reportedly higher in superficial than in deep layers of the superior colliculus (Wichmann and Starke, 1988). This suggests that serotonin is the dominant monoamine affected by amphetamines in the superficial layers of the SC, yet little monoamine transmission occurs within the deeper layers. Further establishing this theory, Dommett et al. (2009) found that therapeutically relevant doses of D-amphetamine and methylphenidate increased the

signal-to-noise ratio in the SC by suppressing weak and preserving strong activations mediated by serotonin via a pre-synaptic mechanism. Furthermore, responses to electrical stimulation of the optic chiasm are also diminished by 5- HT but the response to cortically evoked stimulation is unaffected. Therefore, it has been suggested (Mooney et al., 1996) that 5-HT gates retino-collicular input to the SuG via pre-synaptic receptors as a means of selectively enhancing the relative contribution of cortical input in SuG (at the expense of retino-collicular input) during periods of arousal. Thus, in light of this evidence, Chapter 6 looks at the effects of fluoxetine, another ADHD approved treatments but also a serotonin transporter inhibitor, on SC responses.

6. FLUOXETINE EFFECTS ON COLLICULAR RESPONSES

This chapter describes the findings from an investigation into the effects of intravenously administered fluoxetine on visual superficial layer and auditory deeper layer collicular responses. As stated in the Introduction (Chapter 1), problems within the SC may underlie some of the symptoms found in ADHD and therefore the SC can be investigated in the SHR model of the condition. The key results of an investigation into both visual and auditory responses in the SHR revealed that the SC maybe hyper-responsive to stimuli. This is seen behaviourally towards visual stimuli where the animal has less ability to assess the saliency of, and habituate to, non-salient stimuli and physiologically the SC is more responsive in the SHR. For auditory stimuli there is a lower input into the SC in the SHR suggesting a change in function upstream in these animals. However, the processing in the SC compensates for this reduced input, producing normalised output responses, and behavioural responses. The SHR consistently showed greater onset latency neuronal responses to sensory stimuli, and this is arguably due to a developmental delay in these animals. It was hypothesised that if these differences in the colliculus do indeed underlie symptoms of ADHD, then they should be normalised by treatments successfully prescribed for ADHD, including fluoxetine. Therefore, an investigation was carried out into the effects of fluoxetine on collicular responses in the SHR in comparison to two control strains. The key methods for this chapter are detailed in Chapter 2. The main findings of the study were in line with other research that fluoxetine caused a suppressive effect on both visual and auditory SC responses, similar to the effect of amphetamine (Chapter 5), as the effects of fluoxetine reduced the increased responsiveness in the SHR seen in Chapter 3, potentially by increasing the distractibility threshold to stimuli, and therefore effectively altering the signal-to-noise ratio.

6.1. INTRODUCTION

As previously mentioned, an increase in synaptic availability of the monoamines dopamine, noradrenalin (Azzaro and Rutledge, 1973; Easton et al., 2007) and serotonin (Holmes and Rutledge, 1976; Kuczenski and Segal, 1989) mediates the acute effect of pyschostimulant administration, such as amphetamine, an effective treatment for ADHD. A study found that fluoxetine monotherapy effectively decreased inattention and hyperactivity in children with ADHD and a comorbid non-bipolar mood disorder (Quintana et al., 2007). Similarly, impulse control was improved in rats following treatment with venlafaxine, a serotonin and noradrenalin re-uptake inhibitor (SNRI), but not the dopamine and noradrenalin re-uptake inhibitor, bupropion (Humpston et al., 2013), suggesting that serotonin, and in part noradrenalin, play an important role in the successfully treatment of ADHD. Expansive serotonergic innervation, preferentially innervating the superficial layers is found in the SC (Parent et al., 1981), while restricted noradrenergic (Lindvall and Bjorklund, 1974) and dopaminergic input (Campbell et al., 1991) have been reported. This suggests that serotonin is the dominant monoamine affected by amphetamines in the superficial layers of the SC. A study examining the distribution of serotonin immunoreactivity in the SC in the rat and monkey found serotonergic fibres to be most dense in the SuG in the superficial layers, followed by the InG and DpG in the deeper layers (Ueda et al., 1985). The expansive serotonergic input arises mainly from the dorsal and median raphe nuclei, with terminals located throughout all layers of the SC, but most densely within the Zo and the upper SuG (Mize and Horner, 1989). Stimulation of raphe neurons produces a suppression of visual responses in many superficial SC neurons, or an enhancement of cortical over retinal input to these neurons (Mooney et al., 1996).

Studies have found that fluoxetine, a serotonin selective drug, has therapeutic efficacy in ADHD (Barrickman et al., 1991; Gibson et al., 2006), and ADHD patients have alterations in serotonin metabolism (Hoshino et al., 1985). More specifically, there are two main types

of receptors that could mediate an effect in the SC. 5-HT_{1A} receptors have a postsynaptic localisation and may affect the activity of SC neurons irrespective of the source of input. The 5-HT_{1B} receptors are located preferentially on optic axon terminals and exert presynaptic inhibition of retinotectal inputs (Mooney et al., 1996). A relatively high density of 5-HT_1 binding sites is also found in the SuG. About 70 % were 5-HT_{1B} receptors, a small percentage of 5-HT_{1D} receptor (<6%) and the remaining 30% being 5-HT_{1A} sites (Bruinvels et al., 1993; Boulenguez et al., 1993). Application of 5-HT during blockade of 5-HT_{1A} receptors reduced the amplitude of superficial layer neuron's EPSPs evoked by stimulation of the optic tract from SC slices (Mooney et al., 1996). Therefore, the combined effect of 5-HT at both subtypes ($_{1A}$ and$_{1B}$) would bias SC visual activity toward information received from the corticotectal pathway. It has been suggested (Mooney et al., 1996) that 5-HT gates retino-collicular input to the SuG via pre-synaptic receptors as a means of selectively enhancing the relative contribution of cortical input in SuG (at the expense of retino-collicular input) during periods of arousal. Further evidence of this is seen where intracollicular injection of S-CM-GTNH2, a 5-HT_{1B} and $_{1D}$ agonist, reduced distractibility to peripheral lights in rats (Boulenguez et al., 1995). Further establishing this theory, Dommett et al. (2009) found that therapeutically relevant doses of D–amphetamine and methylphenidate increased the signal-to-noise ratio in the SC by suppressing weak and preserving strong activations mediated by serotonin via a pre-synaptic mechanism. The behavioral effects of these drugs could be linked to a change in the signal-to-noise ratio in the SC by biasing the system towards salient stimuli and consequently leading to a reduction in distractibility, and could underlie key processes involved in the adaptation of the level of reactivity according to the state of arousal of the animal.

Evidence suggests that the SC could be dysfunctional in ADHD and the therapeutic effect of psychostimulants and fluoxetine on decreasing distractibility and improving sustained attention may be mediated via serotonin transmission by an action on the SC. The aim of

the present study is to establish whether fluoxetine does have an inhibitory effect on visual responses, as suggested by other research (Mooney et al., 1996; Dommett et al., 2009), and also to see if fluoxetine has a similar effect on auditory responses within the SC. In light of the findings of amphetamine application seen in Chapter 5, where amphetamine caused a significant decrease in local field potential (amplitude and duration) and multiunit activity visual responses by the final dose for all parameters (increasing onset latency, decreasing amplitude and duration), and as it has already been noted that higher doses of amphetamine work on serotonin and supress visual responses, the aim of the present study is to establish the effects of fluoxetine on visual and auditory collicular responsiveness in the SHR. The aim is to determine whether fluoxetine normalises the SHR strain differences seen in the visual and auditory responses in Chapter 3 and 4. Furthermore, it is theorised that there will be a difference in receptor density of 5-HT_{1A} and 5-HT_{1B} receptors in the SHR strain versus the two control strains which may lead to the behavioural differences seen in this strain.

Hypotheses

10. There will be a significant effect of fluoxetine on visual and auditory responses in the SC in a manner that normalises responses in the SHR, with reference to the WKY and WIS.
11. There will be a difference in receptor density of 5-HT_{1A} and 5-HT_{1B} receptors in the SHR strain than the two control strains (WKY and WIS).

6.2. METHODS

A total of 94 rats were used for the experiments described (32 SHR; 32 WIS; 30 WKY). The animals used for the electrophysiological experiment were not the same animals used for the morphological experiments, as these animals were used for this alone. The weight of the animals immediately prior to experimentation is detailed, by strain and experiment, in

Table 6.1. The normality of the weight data was confirmed using the Kolmogorov–Smirnov test and a One-Way ANOVA was conducted to examine whether there were any strain differences in weight for each type of experiment. This revealed a significant difference in weight between strains for the visual electrophysiological experiment ($F=12.81$; $df=2$; $p=0.0005$). Post hoc (Tukey HSD) analysis revealed the WIS had a significantly greater weight than the WKY ($p=0.002$) and SHR ($p=0.0005$). There was no significant difference between the SHR and the WKY ($p=0.731$). Similar findings were seen in the auditory electrophysiological experiment ($F=27.91$; $df=2$; $p=0.0005$). Post hoc (Tukey HSD) analysis revealed the WIS had a significantly greater weight than the WKY ($p=0.0005$) and SHR ($p=0.0005$). There was no significant difference between the WKY and SHR ($p=0.361$). There was no significant difference in weight between the strains for animals given saline ($F=2.04$; $df=2$; $p=0.186$). There was no significant differences in weight between strains for the immunohistochemistry results (5-HT_{1AR}: $F=2.94$; $df=2$; $p=0.10$; 5-HT_{1BR}: $F=2.68$; $df=2$; $p=0.122$).

Experiment		SHR	WIS	WKY
Vision Drug Electrophysiology	Number of subjects	13	13	11
	Mean weight ± SEM (g)	382.55±9.72	483.24±16.40	399.30±19.61
Vision Saline Electrophysiology	Number of subjects	11	11	11
	Mean weight ± SEM (g)	382.50±9.48	498.90±15.85	354.05±17.09
Saline Electrophysiology	Number of subjects	4	4	4
	Mean weight ± SEM (g)	411.95+5.15	474.08+51.91	431.00+28.14
Immuno-Histochemistry 5-HT_{1AR}	Number of subjects	4	4	4
	Mean weight ± SEM (g)	385.90+8.33	446.72+12.49	432.5+28.14
Immuno-Histochemistry 5-HT_{1BR}	Number of subjects	4	4	4
	Mean weight ± SEM (g)	422.13+25.24	466.00+13.14	427.60+17.54

Table 6.1: The mean ± SEM weights (g) and number of subjects for the experiments within this chapter.

In the previous chapters (Chapter 3 and Chapter 4), differences in sensory responsiveness of the SHR, WIS and WKY were investigated resulting in some baseline differences becoming apparent. In Chapter 5 the investigation included the hooded lister strain (HL) due to their use in previous studies with amphetamine, however, this strain was not used in the present chapter. A small number of animals received saline injections as a control measure and therefore an analysis of this condition was conducted for all three strains. This was followed by an analysis to determine the effects of fluoxetine on each parameter (onset latency, amplitude, duration) using repeated measures ANOVA with STRAIN as the between-subjects factor and DOSE as the within-subjects factor. All saline and drug experiments were carried out using the mid stimulus of the stimulus response curve.

The doses of fluoxetine used in the present chapter are 0.625, 1.25, 2.5, 5, 10 mg/Kg cumulatively. Meyers et al. (2004) found that 80% occupancy of SERT (serotonin transporter) is important for therapeutic effect in humans, while Ginovart et al. (2003) reported 90% SERT occupancy using PET in the striatum, midbrain, and thalamus of cats 30 min after 1 mg/Kg, i.v. fluoxetine administration. Li et al. (2010) also found similar occupancy levels in the rat following 5 mg/Kg s.c. (subcutaneous injection), which would result in bioavailability corresponding to lower i.v. doses. Combined these results, suggest that the lowest two doses of fluoxetine used in the present study are arguably comparable to therapeutically relevant doses in humans but that our higher doses may have exceeded average therapeutic levels. That said, studies have been shown to use concentrations comparable to this study in rats to produce a behavioural effect to fluoxetine, such as a reduction in exploratory location and attention (Dringenberg et al., 2003; LaRoche and Morgan, 2007).

Inclusion criteria

The positions of the 37 visual recordings (13 SHR; 13 WIS; 11 WKY) used in the data analysis were all in the superficial layers of the SC, as shown in the reconstruction of the sections in Figure 6.1 and tabulated in Table 6.2 and Table 6.3. Of the 37 visual recordings used to compare fluoxetine effects, 16 were positioned in the Opticum (Op; 8 SHR; 4 WIS; 4 WKY), while 21 were recorded from the Superficial Grey (SuG; 5 SHR; 9 WIS; 7 WKY). Of these recordings, 25 were positioned in the medial SC (9 SHR; 8 WIS; 8 WKY), and 12 were positioned in the lateral SC (4 SHR; 5 WIS; 3 WKY). Of the 12 visual recordings used to compare saline effects (4 per strain), 5 were positioned in the Superficial Grey (2 SHR; 1 WIS; 2 WKY), and 7 were positioned in the Opticum (2 SHR; 3 WIS; 2 WKY). Of these saline recordings, 6 were positioned in the medial SC (3 SHR; 1 WIS; 2 WKY), and 6 were positioned in the lateral SC (1 SHR; 3 WIS; 2 WKY). Chi-square analysis showed there was no significant association between the positioning of the electrodes and strain in terms of anterior-posterior positioning (χ^2=0.345; df=4; p=0.987); medial-lateral positioning (χ^2=0.37; df=2; p=0.833) or superficial layer positioning (χ^2=2.81; df=2; p=0.245).

Co-ordinates From Bregma	Layer	SHR N=13	WIS N=13	WKY N=11
-5.8mm	Zonal Layer	0	0	0
	Superficial Grey	1	2	2
	Opticum	2	1	1
-6.3mm	Zonal Layer	0	0	0
	Superficial Grey	3	5	4
	Opticum	4	3	2
-6.8mm	Zonal Layer	0	0	0
	Superficial Grey	1	2	1
	Opticum	2	0	1

Table 6.2: The anterior-posterior and layer positioning of the electrodes for the visual responses within the superficial layers of the superior colliculus for each strain. Chi-square analysis revealed no significant associations.

	SHR	WIS	WKY
Medial Recordings	9	8	8
Lateral Recordings	4	5	3

Table 6.3: The medial-lateral positioning of the electrodes for the visual responses. Chi-square analysis revealed no significant associations.

The positions of the 33 auditory recordings (11 SHR; 11 WIS; 11 WKY) used in the data analysis were all in the deeper layers of the SC as shown in the reconstruction of the sections in Figure 6.1 and tabulated in Table 6.4 and 6.5. Of the 33 auditory responses used for stimulus response analysis, 22 were positioned in the Intermediate Grey (InG; 5 SHR; 8 WIS; 9 WKY), 1 was recorded from the Intermediate White (InW; 1 WKY) and 1 was recorded in the Deep White (DpW; 1 SHR) with the remaining 9 responses were recorded in the Deep Grey (DpG; 5 SHR; 3 WIS; 1 WKY). Of these recordings, 23 were positioned in the medial SC (8 SHR; 8 WIS; 7 WKY), and 10 were positioned in the lateral SC (3 SHR; 3 WIS; 4 WKY). Of the 12 auditory recordings used to compare saline effects (4 per strain), 5 were positioned in the Intermediate Grey (InG; 2 SHR; 1 WIS; 2 WKY), and 7 were positioned in the Deep Grey (DpG; 2 SHR; 3 WIS; 2 WKY). Of these saline recordings, 8 were positioned in the medial SC (2 SHR; 3 WIS; 3 WKY), and 4 were positioned in the lateral SC (2 SHR; 1 WIS; 1 WKY). Chi-square analysis showed there were no significant associations between the positioning of the electrodes and strain with regards to anterior-posterior positioning (χ^2=1.39; df=4; p=0.850), medial-lateral positioning (χ^2=0.29; df=2; p=0.866), or deeper layer positioning (χ^2=7.85; df=6; p=0.249).

Co-ordinates From Bregma	Layer	SHR N=11	WIS N=11	WKY N=11
-5.8mm	Intermediate Grey	0	2	2
	Intermediate White	0	0	0
	Deep Grey	2	1	1
	Deep White	0	0	0
-6.3mm	Intermediate Grey	3	4	6
	Intermediate White	0	0	0
	Deep Grey	1	2	0
	Deep White	1	0	0
-6.8mm	Intermediate Grey	2	2	1
	Intermediate White	0	0	1
	Deep Grey	2	0	0
	Deep White	0	0	0

Table 6.4: The anterior-posterior and layer positioning of the electrodes for the auditory responses within the deeper layers of the superior colliculus for each strain. Chi-square analysis revealed no significant associations.

	SHR	WIS	WKY
Medial Recordings	8	8	7
Lateral Recordings	3	3	4

Table 6.5: The medial-lateral positioning of the electrodes for the auditory responses. Chi-square analysis revealed no significant associations.

Figure 6.1: Reconstructed plots of recording sites in the SC. During collicular recordings, SHR recording sites are shown in black, WKY recording sites are shown in green, Wistar recording sites are shown in blue. Adapted from Paxinos and Watson (1998).

Saline effects on visual and auditory responses

Equivalent cumulative doses of saline caused no significant effect on visual or auditory responses for any parameter (see Table 6.6). There were no main effects of DOSE or STRAIN or DOSE x STRAIN interaction for any parameter.

Experiment	Parameter	Main Effect of DOSE	Interaction	Main effect of STRAIN
LFP vision response	**Onset Latency**	F=1.11; df=2.47, 0.11; p=0.358	F=1.23; df=4.93, 0.22; p=0.328	F=1.51; df=2, 0.25; p=0.273
	Amplitude	F=1.54; df=3.06, 0.15; p=0.227	F=0.58; df=6.11, 0.11; p=0.749	F=0.93; df=2, 0.17; p=0.431
	Duration	F=2.46; df=3.10, 0.22; p=0.082	F=1.35; df=6.20, 0.23; p=0.269	F=0.78; df=2, 0.15; p=0.486
Multiunit activity vision response	**Onset Latency**	F=1.07; df=5, 0.11; p=0.388	F=1.18; df=10, 0.21; p=0.326	F=0.19; df=2, 0.04; p=0.828
	Amplitude	F=0.81; df=1.92, 0.08; p=0.456	F=1.01; df=3.83, 0.18; p=0.426	F=0.06; df=2, 0.01; p=0.940
	Duration	F=1.22; df=5, 0.12; p=0.314	F=0.93; df=10, 0.17; p=0.514	F=0.00; df=2, 0.00; p=0.996
LFP auditory response	**Onset Latency**	F=1.42; df=2.75, 0.14; p=0.261	F=0.63; df=5.51, 0.12; p=0.695	F=0.27; df=2, 0.06; p=0.770
	Amplitude	F=0.90; df=2.13, 0.09; p=0.427	F=1.45; df=4.25, 0.24; p=0.254	F=1.44; df=2, 0.24; p=0.287
	Duration	F=1.43; df=5, 0.14; p=0.232	F=0.53; df=10, 0.11; p=0.859	F=0.03; df=2, 0.01; p=0.970
Multiunit activity auditory response	**Onset Latency**	F=0.60; df=1.72, 0.06; p=0.539	F=1.05; df=3.44, 0.19; p=0.405	F=0.95; df=2, 0.18; p=0.422
	Amplitude	F=1.95; df=5, 0.18; p=0.105	F=1.44; df=10, 0.24; p=0.193	F=1.88; df=2, 0.29; p=0.208
	Duration	F=1.29; df=5, 0.13; p=0.285	F=1.31; df=10, 0.23; p=0.254	F=0.10; df=2, 0.02; p=0.902

Table 6.6: The statistical analysis of the saline effects at each parameter.

6.3.1. EFFECTS OF FLUOXETINE ON VISUALLY-EVOKED LOCAL FIELD POTENTIALS

As previously mentioned in Section 2.3.5, the data was filtered into LFP and multiunit activity. Visual local field potentials are considered as the synchronised input into the recording space, in this case the superficial layers of the superior colliculus. As high frequencies are filtered out, slower frequencies representing the postsynaptic potential, (i.e. excitatory postsynaptic potentials and inhibitory postsynaptic potentials) were kept for analysis.

Onset latency

Onset latency of the visual response to contralateral visual stimulation for each of the three strains following administration of cumulative doses of fluoxetine is shown in Figure 6.2. There was a significant main effect of DOSE (F=2.59; df=3.45, 0.07; p=0.048). Within-subjects contrasts showed that there was a significant increase in onset latency at dose 2.5 mg/Kg (F=6.72; df=1, 0.17; p=0.014), 5 mg/Kg (F=4.51; df=1, 0.12; p=0.041) and 10 mg/Kg (F=9.20; df=1, 0.21; p=0.005) compared to baseline. There were no significant differences of any other doses relative to baseline. There was no main effect of STRAIN (F=0.87; df=2, 0.05; p=0.427). There was no significant STRAIN x DOSE interaction (F=1.38; df=6.89, 0.08; p=0.221).

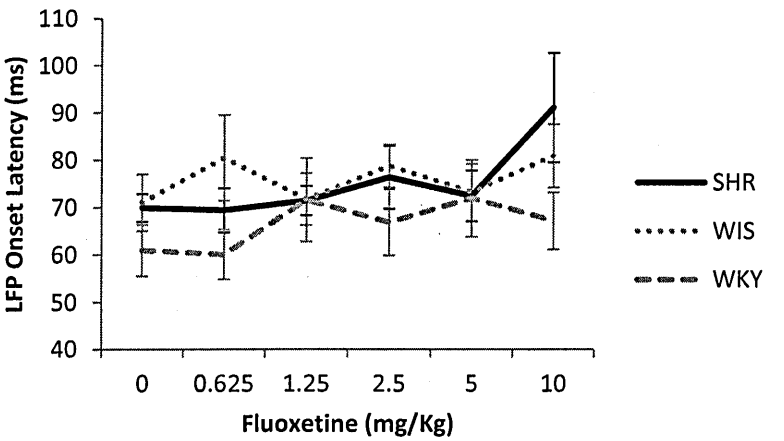

Figure 6.2: The mean ± SEM visual response LFP onset latency of the three strains over the increasing fluoxetine dose showing a significant main effect of DOSE, with an increasing onset latency as dose increased. There was no main effect of STRAIN or DOSE x STRAIN interaction.

Amplitude

Amplitude of the visual response to contralateral visual stimulation for each of the three strains following administration of cumulative doses of fluoxetine is shown in Figure 6.3. There was no main effect of DOSE (F=2.49; df=2.23, 0.07; 0.084). There was a main effect of STRAIN (F=4.86; df=2, 0.22; p=0.014). Post hoc (Tukey HSD) analysis revealed that the WKY had a larger amplitude than the SHR (p=0.023) and WIS (p=0.029), there was no significant difference between the SHR and WIS (p=0.996). There was no significant STRAIN x DOSE interaction (F=0.53; df=4.46, 0.03; df=0.733).

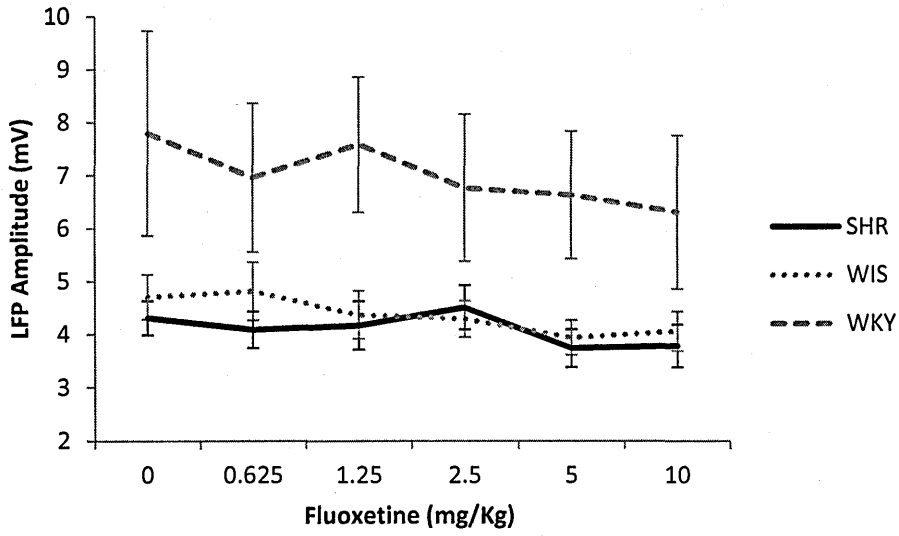

Figure 6.3: The mean ± SEM visual response LFP amplitude of the three strains over the increasing fluoxetine dose showing a no main effect of DOSE. There was a main effect of STRAIN where the WKY had larger amplitude than the SHR and WIS. There was no DOSE x STRAIN interaction.

Duration

Duration of the response to contralateral visual stimulation for each of the three strains following administration of cumulative doses of fluoxetine is shown in Figure 6.4. There was a main effect of DOSE (F=7.10; df=3.48, 0.17; p=0.0005). Within-subjects contrasts showed that there was a significant decrease in duration with 1.25 mg/Kg (F=4.86; df=1, 0.13; p=0.034), 2.5 mg/Kg (F=5.51; df=1, 0.14; p=0.025), 5 mg/Kg (F=6.73; df=1, 0.17; p=0.014) and 10 mg/Kg (F=22.69; df=1, 0.40; p=0.0005) compared to baseline, there were no significant difference for the lowest dose. There was no main effect of STRAIN (F=0.71; df=2, 0.04; p=0.499). There was no significant STRAIN x DOSE interaction (F=0.84; df=6.95, 0.05; p=0.557).

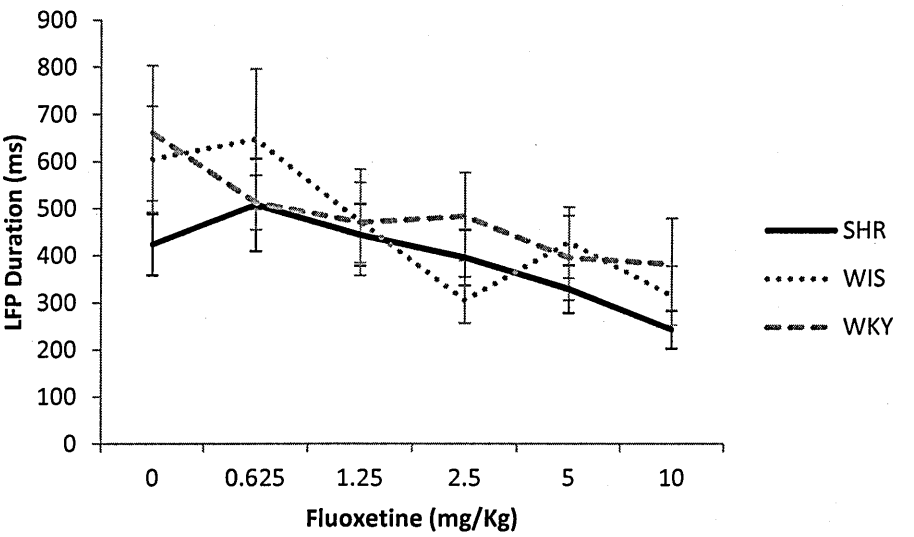

Figure 6.4: The mean ± SEM visual response LFP duration of the three strains over the increasing fluoxetine dose showing a main effect of DOSE, as the dose increased the duration decreased. There was no main effect of STRAIN and there was no DOSE x STRAIN interaction.

To summarise, fluoxetine had a suppressive effect on local-field potential visual responses, causing significant increases in onset latency, and significant decreases in response duration. The SHR had significantly smaller LFP response amplitude than the WKY, the WKY also had greater response amplitude than the WIS, yet all the strains reacted to the drug similarly. The only strain difference found in Chapter 3 was that the SHR had decreased response duration in

comparison to one control strain, as there was no interaction for this parameter it suggests that fluoxetine did not normalise the strain differences seen in the SHR in Chapter 3.

6.3.2. EFFECTS OF FLUOXETINE ON VISUALLY-EVOKED MULTIUNIT ACTIVITY RESPONSES

As previously mentioned in Section 2.3.5, the vision data was filtered into LFP and multiunit activity. The visual multiunit activity data represents the output from the area, in this case the superficial layers of the superior colliculus. The fast frequencies are mostly caused by the short inward and outward currents of action potentials, representing the spike activity of neurons and were kept for analysis.

Onset latency

Multiunit activity onset latency in response to contralateral visual stimulation for each of the three strains following administration of cumulative doses of fluoxetine is shown in Figure 6.5. There was no main effect of DOSE (F=0.44; df=2.39, 0.01; p=0.681). There was no main effect of STRAIN (F=1.33; df=2, 0.07; p=0.279). There was no significant STRAIN x DOSE interaction (F=0.79; df=4.77, 0.04; p=0.552).

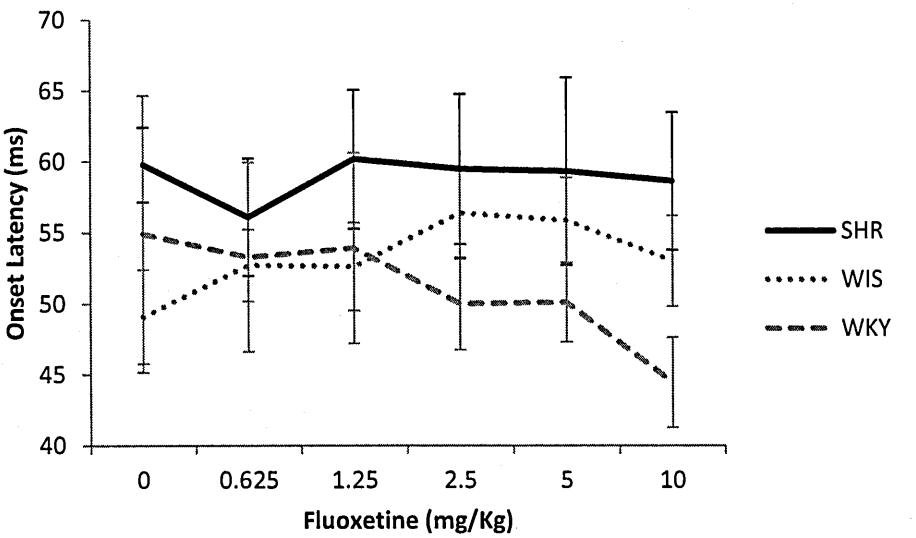

Figure 6.5: The mean ± SEM visual response multiunit activity onset latency of the three strains over the increasing fluoxetine dose. There was no main effect of DOSE or STRAIN and there was no DOSE x STRAIN interaction.

Amplitude

Multiunit activity amplitude in response to contralateral visual stimulation for each of the three strains following administration of cumulative doses of fluoxetine is shown in Figure 6.6. There was a main effect of DOSE (F=4.30; df=2.95, 0.11; p=0.007). Within-subjects contrasts showed that there was a significant decrease in amplitude with 2.5 mg/Kg (F=4.87; df=1, 0.13; p=0.034), 5 mg/Kg (F=10.88; df=1, 0.24; p=0.002) and 10 mg/Kg (F=6.61; df=1, 0.16; p=0.015) compared to baseline, there were no significant differences of any other doses relative to baseline. There was no main effect of STRAIN (F=1.10; df=2, 0.06; p=0.346). There was no significant STRAIN x DOSE interaction (F=0.58; df=5.90, 0.03; p=0.742).

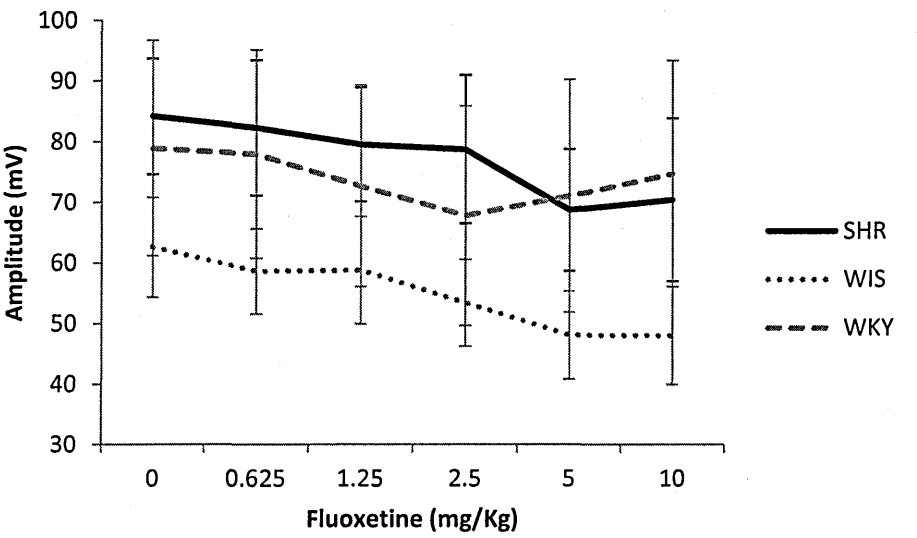

Figure 6.6: The mean ± SEM visual response multiunit activity amplitude of the three strains over the increasing fluoxetine dose showing a main effect of DOSE, as the dose increased the amplitude decreased. There was no main effect of STRAIN and there was no DOSE x STRAIN interaction.

Duration

Multiunit activity duration in response to contralateral visual stimulation for each of the three strains following administration of cumulative doses of fluoxetine is shown in Figure 6.7. There was a main effect of DOSE (F=4.22; df=3.46, 0.11; p=0.005). Within-subjects contrasts showed that there was a significant decrease in duration with 10 mg/Kg (F=11.84; df=1, 0.26; p=0.002) compared to baseline, there were no significant differences of any other doses relative to baseline. There was no main effect of STRAIN (F=0.34; df=2, 0.20; p=0.711). There was no significant STRAIN x DOSE interaction (F=1.04; df=6.91, 0.06; p=0.408).

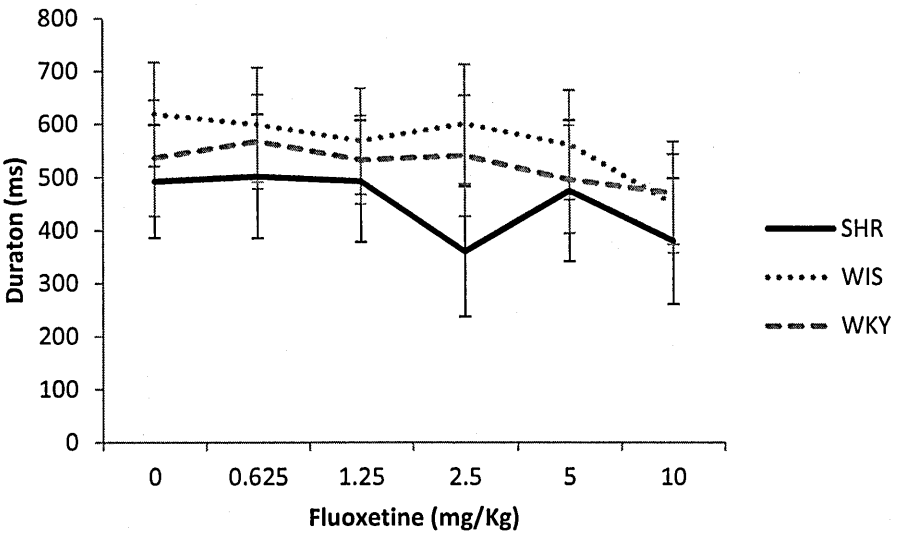

Figure 6.7: The mean ± SEM visual response multiunit activity duration of the three strains over the increasing fluoxetine dose showing a main effect of DOSE, as the dose increased the duration decreased. There was no main effect of STRAIN and there was no DOSE x STRAIN interaction.

To summarise, fluoxetine caused a suppressive effect on multiunit activity visual responses, causing a significant decrease in amplitude and response duration. There were no strain differences, and all strains reacted to the drug similarly. The only strain difference found in Chapter 3 was that the SHR had increased response onset latency in comparison to one control strain, and the SHR had increased amplitude to light in comparison to the two control strains.

As there was no DRUG x STRAIN interaction for these parameters it suggests that fluoxetine did not normalise the strain differences seen in the SHR in Chapter 3.

6.3.3. EFFECTS OF FLUOXETINE ON AUDITORY-EVOKED LOCAL FIELD POTENTIALS

As previously mentioned in Section 2.3.5, the data was filtered into LFP and multiunit activity. Auditory local field potentials are considered as the synchronised input into the recording space, in this case the deeper layers of the superior colliculus.

Onset latency

LFP onset latency in response to auditory stimulation for each of the three strains following administration of cumulative doses of fluoxetine is shown in Figure 6.8. There was no main effect of DOSE (F=1.91; df=3.26, 0.06; p=0.129). There was no main effect of STRAIN (F=2.77; df=2, 0.16; p=0.079). There was no significant STRAIN x DOSE interaction (F=0.73; df=6.51, 0.05; p=0.641).

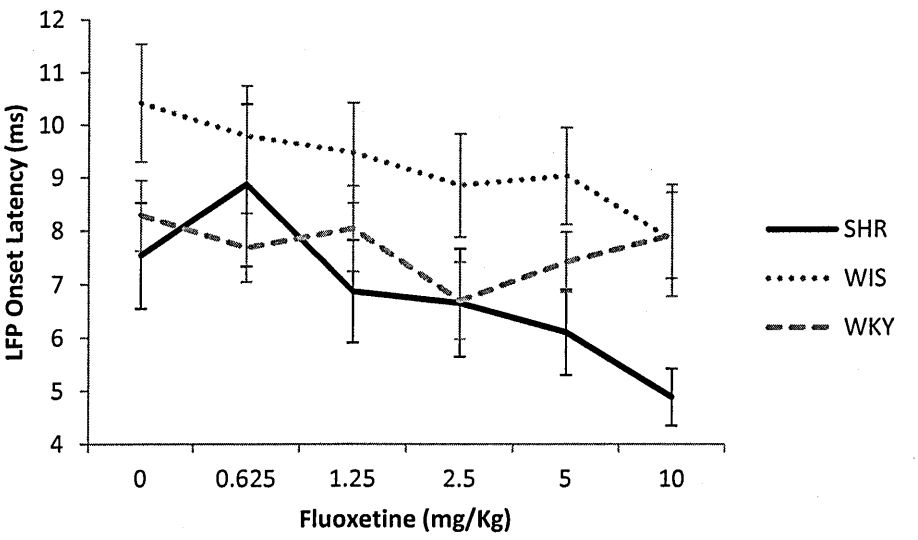

Figure 6.8: The mean ± SEM auditory response LFP onset latency of the three strains over the increasing fluoxetine dose showing there was no main effect of DOSE or STRAIN and there was no DOSE x STRAIN interaction.

Amplitude

LFP amplitude in response to auditory stimulation for each of the three strains following administration of cumulative doses of fluoxetine is shown in Figure 6.9. There was a main effect of DOSE (F=5.92; df=3.28, 0.17; p=0.001). Within-subjects contrasts showed that there was a significant increase in amplitude with 1.25 mg/ Kg (F=9.79; df=1, 0.25; p=0.004), 2.5 mg/Kg (F=23.88; df=1, 0.44; p=0.0005), 5 mg/Kg (F=5.72; df=1, 0.16; p=0.023) and 10 mg/Kg (F=17.85; df=1, 0.37; p=0.0005) compared to baseline, there were no significant difference for the lowest dose. There was no main effect of STRAIN (F=2.55; df=2, 0.15; p=0.095). There was no significant STRAIN x DOSE interaction (F=1.84; df=6.55, 0.11; p=0.093).

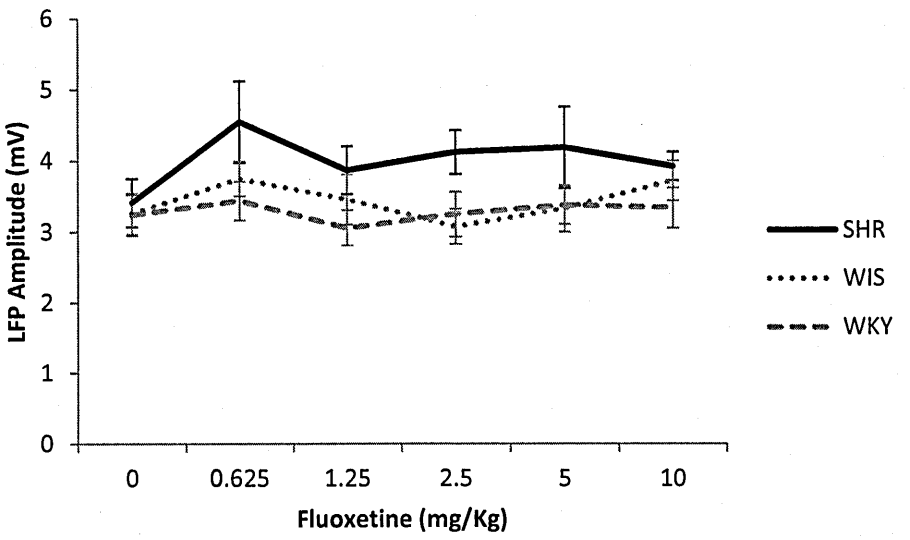

Figure 6.9: The mean ± SEM auditory response LFP amplitude of the three strains over the increasing fluoxetine dose showing there was a main effect of DOSE. Increasing doses of fluoxetine caused an increase in amplitude. There was no main effect of STRAIN and there was no DOSE x STRAIN interaction.

Duration

LFP duration in response to auditory stimulation for each of the three strains following administration of cumulative doses of fluoxetine is shown in Figure 6.10. There was no main effect of DOSE (F=0.42; df=3.13, 0.01; p=0.748). There was a main effect of STRAIN (F=7.12; df=2, 0.32; p=0.003). Post hoc (Tukey HSD) analysis revealed the SHR had a significantly longer duration than the WKY (p=0.002), and a trend towards a significantly longer duration than the WIS (p=0.055). There was no significant STRAIN x DOSE interaction (F=0.82; df=6.26, 0.05; p=0.563).

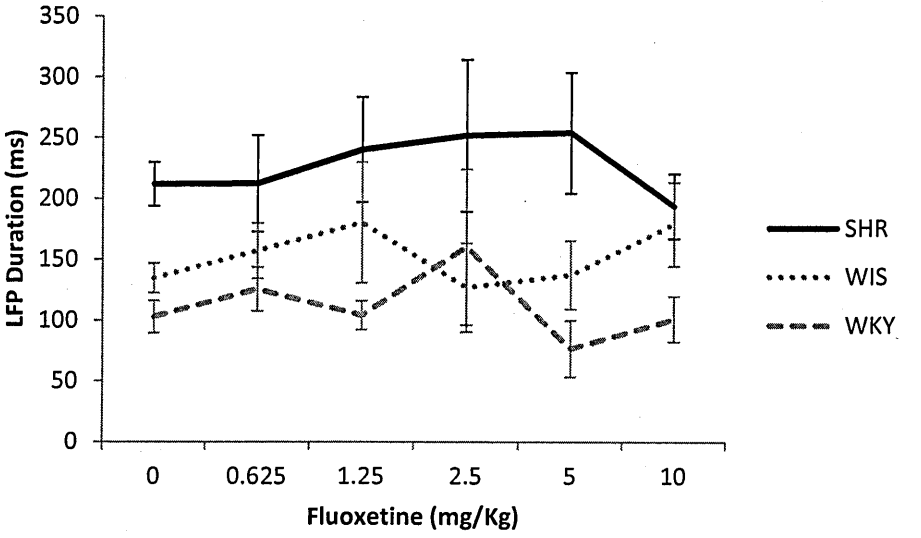

Figure 6.10: The mean ± SEM auditory response LFP duration of the three strains over the increasing fluoxetine dose showing there was no main effect of DOSE. There was a main effect of STRAIN, the SHR had a significantly longer duration than the WKY and a trend towards a significantly longer duration than the WIS. There was no DOSE x STRAIN interaction.

To summarise, fluoxetine caused a excitatory effect on local-field potential auditory responses, causing a significant increase in response amplitude with all strains reacting to the drug similarly. Strain differences between the SHR and the control strains were seen for all parameters in Chapter 4 (lower amplitude, decrease duration and trend towards significantly greater onset latency) as there was no DRUG x STRAIN interaction for any parameter it suggests that fluoxetine did not normalise the strain differences seen in the SHR in Chapter 4.

6.3.4. EFFECTS OF FLUOXETINE ON AUDITORY-EVOKED MULTIUNIT ACTIVTY RESPONSES

As previously mentioned in Section 2.3.5, the vision data was filtered into LFP and multiunit activity. The auditory multiunit activity data represents the output from the area, in this case the deeper layers of the superior colliculus.

Onset latency

Multiunit activity onset latency in response to auditory stimulation for each of the three strains following administration of cumulative doses of fluoxetine is shown in Figure 6.11. There was no main effect of DOSE ($F=1.09$; $df=2.75, 0.04$; $p=0.356$). There was no main effect of STRAIN ($F=0.24$; $df=2, 0.02$; $p=0.786$).There was no significant STRAIN x DOSE interaction ($F=1.5$; $df=5.49, 0.07$; $p=0.341$).

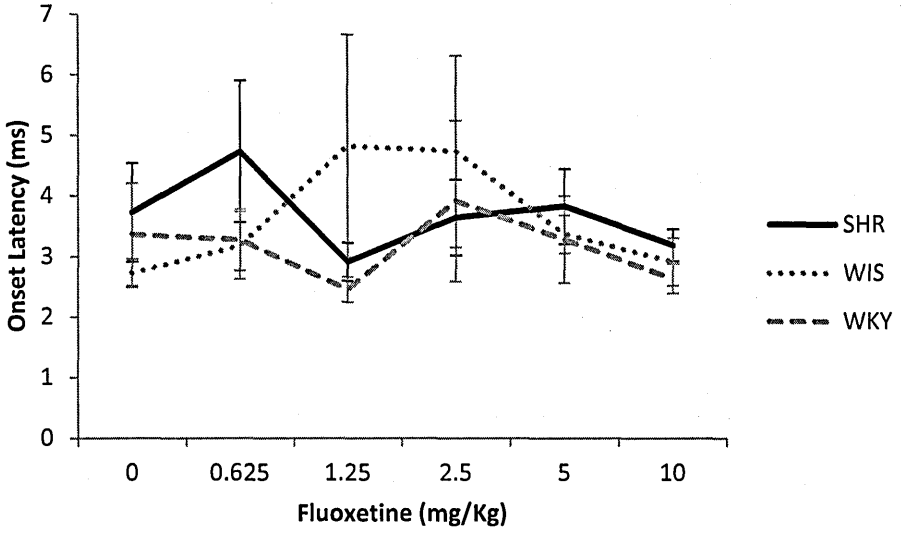

Figure 6.11: The mean ± SEM auditory response multiunit activity onset latency of the three strains over the increasing fluoxetine dose showing there was no main effect of DOSE. There was no main effect of STRAIN. There was no DOSE x STRAIN interaction.

Amplitude

Multiunit activity amplitude in response to auditory stimulation for each of the three strains following administration of cumulative doses of fluoxetine is shown in Figure 6.12. There was a main effect of DOSE (F=10.07; df=2.99, 0.25; p=0.0005). Within-subjects contrasts showed that there was a significant decrease in amplitude with 0.625 mg/Kg (F=10.83; df=1, 0.265; p=0.003), 1.25 mg/ Kg (F=5.34; df=1, 0.15; p=0.028), 2.5 mg/Kg (F=13.57; df=1, 0.31; p=0.001), 5 mg/Kg (F=13.28; df=1, 0.31; p=0.001) and 10 mg/Kg (F=21.06; df=1, 0.41; p=0.0005) compared to baseline. There was no main effect of STRAIN (F=0.16; df=2, 0.01; p=0.853). There was no significant STRAIN x DOSE interaction (F=1.36; df=5.97, 0.08; p=0.242).

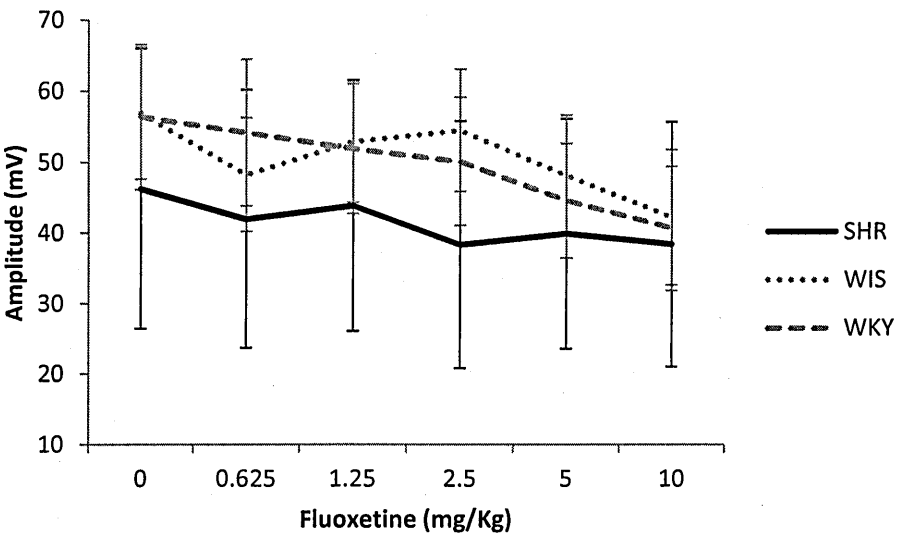

Figure 6.12: The mean ± SEM auditory response multiunit activity amplitude of the three strains over the increasing fluoxetine dose showing there was a main effect of DOSE, as the dose of fluoxetine increased the amplitude decreased. There was no main effect of STRAIN. There was no DOSE x STRAIN interaction.

Duration

Multiunit activity duration in response to auditory stimulation for each of the three strains following administration of cumulative doses of fluoxetine is shown in Figure 6.13. There was no main effect of DOSE (F=0.56; df=2.53, 0.02; p=0.613). There was no main effect of STRAIN (F=2.07; df=2, 0.12; p=0.144). There was no significant STRAIN x DOSE interaction (F=1.25; df=5.07, 0.08; p=0.296).

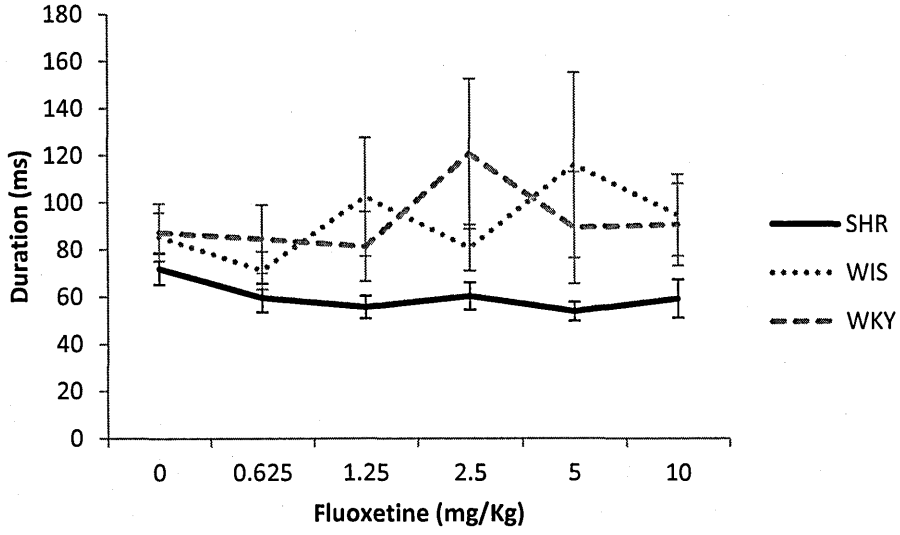

Figure 6.13: The mean ± SEM auditory response multiunit activity duration of the three strains over the increasing fluoxetine dose showing there was no main effect of DOSE or STRAIN. There was no DOSE x STRAIN interaction.

To summarise, fluoxetine caused a suppressive effect on multiunit activity auditory responses, causing a significant decrease in response amplitude. There were no strain differences, and all strains reacted to the drug similarly. The only strain difference found in Chapter 4 was that the SHR had decreased response onset latency in comparison to the two control strain, as there was no interaction for this parameter it suggests that fluoxetine did not normalise the strain differences seen in the SHR in Chapter 4.

6.3.5. QUANTIFICATION OF DAB STAINING

Serotonin$_{1A}$ receptor density

The receptor density of $5HT_{1AR}$ was calculated for both the superficial and deeper layers of the SC in all three strains and is shown in Figure 6.14. The density was compared across all three strains using a One-Way ANOVA for both regions. There was no significant difference in density between strains for either the superficial layers (F=3.34; df=2, 4.26; p=0.082) or deeper layers (F=0.721; df=2, 4.26; p=0.512).

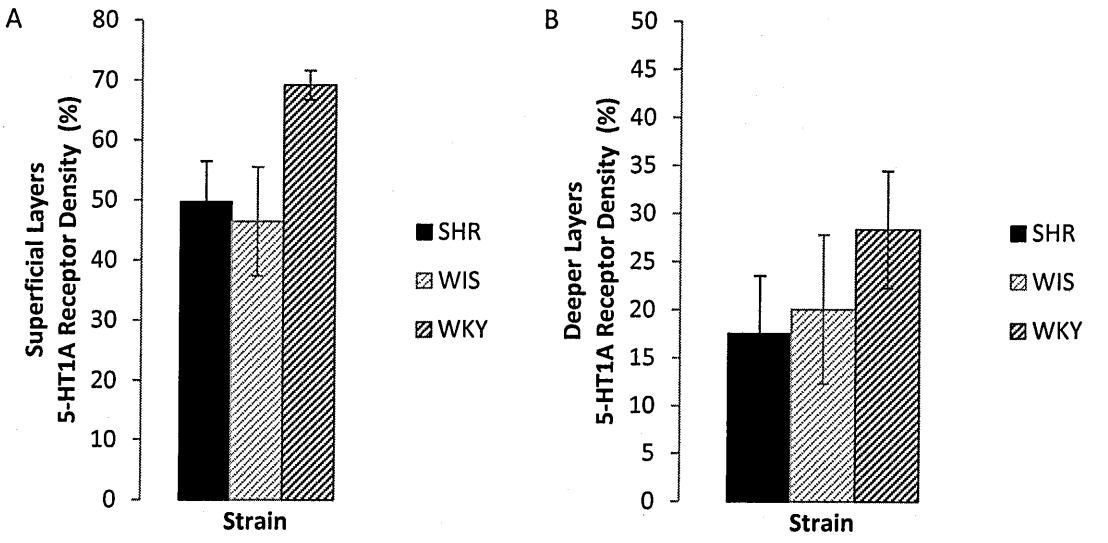

Figure 6.14: The receptor density of $5HT_{1AR}$ in the superficial and deeper layers of the three strains (N=4 per strain). There were no significant differences.

Serotonin$_{1B}$ receptor density

The receptor density of $5HT_{1BR}$ was calculated for both the superficial and deeper layers of the SC in all three strains and is shown in Figure 6.15. The density was compared across all three strains using a One-Way ANOVA for both regions. There was no significant difference in density between strains for either the superficial layers (F=0.68; df=2, 4.26; p=0.530) or deeper layers (F=0.41; df=2, 4.26; p=0.673).

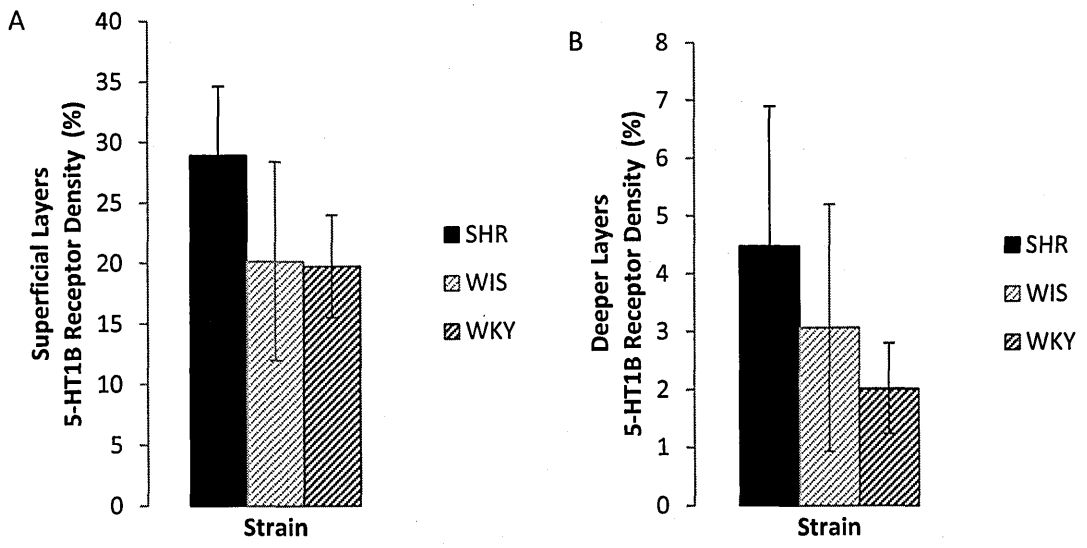

Figure 6.15: The receptor density of $5HT_{1BR}$ in the superficial and deeper layers of the three strains (N=4 per strain). There were no significant differences

To summarise, despite the SHR having a higher 5-HT$_{1B}$ receptor density than the two control strains, neither the percentage density of 5-HT$_{1A}$ or 5-HT$_{1B}$ receptors were significantly different in either the superficial or deeper SC layers of these strains.

6.4. DISCUSSION

Fluoxetine had a suppressive effect on visual responses, causing a significant increase in onset latency and decrease in duration of local field potential visual responses, and a significant decrease in multiunit activity amplitude and duration. All strains reacted

similarly to the drug, and the strain differences in the SHR seen in Chapter 3 (increased amplitude and greater onset latency multiunit responses) were not normalised to comparable control baseline values by fluoxetine. Interestingly, the WKY had a larger LFP vision amplitude than the SHR and WIS, which was not seen in Chapter 3.

Fluoxetine caused a significant increase in auditory local-field potential amplitudes, but a significant decrease in multiunit activity auditory amplitude, suggesting an excitatory effect on SC inputs, yet an overall suppressive effect on SC processing of auditory responses. The SHR had a significantly longer LFP duration than the WKY and a trend towards a significantly longer duration than the WIS. All strains reacted similarly to the drug, and the strain differences in the SHR seen in Chapter 4 (lower LFP amplitude and greater multiunit response onset latency) were not normalised to comparable control baseline values by fluoxetine. Interestingly, the SHR had a significantly longer LFP auditory duration than the WKY which was not seen in Chapter 4.

There were no significant differences in the amount of $5HT_{1AR}$ or $5HT_{1BR}$ in this study, however, the variance of this data was quite large and the sample size quite small so this part of the study may have lacked sufficient statistical power. An increase in sample size may be useful in determining if there are any receptor density differences seen in the SC for these strains. However, the findings of non-significant differences in receptor densities are in line with the physiological finding that all animals responded similarly to the drug and the latter data were based on a larger sample size.

The results found in this chapter are in line with other research findings a suppressive effect of serotonin on SC responses (Mooney et al., 1996; Dommett et al., 2009). Activation of the $5\text{-}HT_{1B}$ receptor on retinal ganglion cell terminals in the SC is known to reduce

glutamate neurotransmission in adult hamsters (Mooney et al., 1994). Similarly, application of 5-HT during blockade of 5-HT_{1A} receptors with spiperone (a 5-HT_{1A}, 5-HT_{2A}, 5-HT_7, D_2 receptor antagonist) reduced the amplitude of superficial layer neurons EPSPs evoked by stimulation of the optic tract from SC slices (Mooney et al., 1996). Application of 5-HT by iontophoresis, micropressure or by electrical stimulation of the dorsal raphé nucleus producing endogenous release of 5-HT, greatly suppressed visual activity in SC neurons during extracellular *in vivo* recordings (Mooney et al., 1996). Furthermore, 5-HT application produced a greater suppression of visual SC responses to electrical stimulation of the optic chiasm (92.2% reduction) than the suppression seen evoked by visual cortex stimulation (32.2% reduction) (Mooney et al., 1996). Therefore, the combined effect of 5-HT at both subtypes ($_{1A}$ and $_{1B}$) would bias SC visual activity toward information received from the corticotectal pathway. In light of this it is further suggested (Mooney et al., 1996) that 5-HT gates retino-collicular input to the SuG via pre-synaptic receptors as a means of selectively enhancing the relative contribution of cortical input in SuG (at the expense of retino-collicular input) during periods of arousal.

Boulenguez et al. (1995) introduced peripheral lights at the mid-points of the animals' run down a run way to analyse distraction following drug treatment. In the weaker distracting condition (unilateral distractor) only, distraction indexes were decreased and therefore the animals were less distracted by the peripheral light, following intracollicular injection of S-CM-GTNH2, a 5-HT_{1B} and $_{1D}$ agonist. Similarly, when treated with S-CM-GTNH 2, rats were found to be more distracted by the stronger (bilateral) distractors than by the unilateral ones. It was suggested that 5-HT, via 5-$HT_{1B\text{-}1D}$ receptors may cause an increase of the visual distractibility threshold by modulating directly the transmission of the primary visual signal.

As previously mentioned, Dommett et al. (2009) found that therapeutically relevant doses of D-amphetamine and methylphenidate increased the signal-to-noise ratio in the SC by suppressing weak and preserving strong activations mediated by serotonin via a pre-synaptic mechanism. It has been shown that serotonin reduces the signal-to-noise ratio in the somatosensory cortex (Waterhouse et al., 1986) and thalamus (Funke and Eysel, 1993), yet not by altering the weak and strong signal relationship but by suppressing spontaneous background activity. The behavioral effects of these drugs could be linked to a change in the signal-to-noise ratio effect mediated in the SC by biasing the system towards salient stimuli and consequently leading to a reduction in distractibility, and could underlie key processes involved in the adaption of the reactivity according to the state of arousal of the animal.

From a medical standpoint, as previously mentioned fluoxetine has been shown to be therapeutically effective in the treatment of ADHD. Interestingly, despite no receptor density differences being seen in the present results, evidence does suggest a possible link between an increased 5-HT_{1B} receptor density and behaviours seen in the SHR. 5-HT_{1B} receptors are linked to aggression (de Boer and Koolhass, 2005), the SHRs are noted to be an overly aggressive strain in comparison to control strains (Toot et al., 2004). 5-HT_{1B} receptors are also localised on blood vessels, and linked to vasoconstriction. The "triptans" are a drug class useful as abortive medication for the treatment of acute migraine headaches. They are very effective medications that bind to 5-HT_{1B} and 5-HT_{1D} receptors in cranial vessels, which lead to vasoconstriction and decreased release of neuropeptides involved in "sterile inflammation" (Ahn and Basbaum, 2005). This could suggest the differences seen in fluoxetine are due to changes in blood pressure, notably already a problem in the SHR, yet as there were no strain differences in the effects of the drug on responses, this is unlikely to be the case.

Further evidence of a greater density of (5-HT_{1B} and $_{1D}$) receptors, despite not being significant in the results in this chapter, is that following intracolliculuar injection of S-CM-GTNH2 (5-HT_{1B} and $_{1D}$ agonist), Boulenguez et al., (1995) found that animals exhibited an erratic running style, involving side-to-side movements of the head, without a change in the overall accuracy of their locomotor trajectories. The motor effects observed in Boulenguez et al., (1995) were due to a ventral spread of the drug into deeper laminae, and indicates the physiological importance of the (even) low density of 5-HT1B receptors in deeper collicular laminae involved in the organisation of coordinated orienting movements (Foreman, 1983). The most likely output for such cells is via the predorsal bundle, which has inhibitory influences on brainstem nuclei involved in motor control and locomotor movement (Boulenguez et al., 1995).

5-HT_{1B} and $_{1D}$ receptor are involved in the inhibition of retinal afference to the SC by serotonin. This type of neuromodulatory control by serotonin could be generalised to other primary and secondary sensory inputs, as shown by several authors (Alhaider and Wilcox, 1993; Hegerl and Juckel, 1993; Waterhouse et al., 1990), and supports the findings in the auditory results data in this chapter.

As previously mentioned, all animals did respond to fluoxetine in the same way. Similar findings were seen in Chapter 5 in regards to amphetamine application. Amphetamine caused a significant decrease in local field potential and multiunit activity visual responses by the final dose for all parameters (increasing onset latency, decreasing amplitude and duration). It has already been noted that higher doses of amphetamine work on serotonin and supress visual responses. Interestingly, unlike amphetamine (see Section 5.3), fluoxetine has shown no positive effects on attention or cognitive enhancement in healthy individuals. Allen et al. (1988) found no effect in psychomotor and memory tests following

the administration of a 40 mg dose of fluoxetine to healthy volunteers each morning for 1 week. In contrast, fluoxetine has been shown to have negative effects on attention. It caused a decrease in sustained attention, as well as a decrease concentration in healthy volunteers over a 3 week trial (Ramaekers et al., 1995). Albeit not normalised to comparable control baseline values, similar to the effect of amphetamine, fluoxetine reduced the increased responsiveness in the SHR seen in Chapter 3. Therefore, potentially increasing the distractibility threshold to stimulus, and therefore effectively altering the signal-to-noise ratio. Fluoxetine's mechanism of action in these animals and ADHD sufferers could be to normalise differences in the SC visual responses and thus normalise behavioural differences. Fluoxetine treatment efficacy might be gender specific, at least in rats, as a study found that treatment of fluoxetine improved visual attention in a task in females, but produced a reduction in attentional performance in male rats (LaRoche and Morgan, 2007), it suggests that fluoxetine may be a more successful treatment for female suffers.

7. FINAL DISCUSSION AND CONCLUSIONS

The previous chapters have outlined the theoretical and experimental background to the work presented and discussed the results of the studies conducted. The present chapter draws together the major findings and discusses the implications of these for the main theories of ADHD and offers support for the novel theory of the disorder proposed by Overton (2008). In addition to this, the limitations of the current work are discussed and future directions are suggested.

7.1. PRINCIPAL FINDINGS

The aim of the work presented in this thesis was to examine any behavioural, physiological and gross morphological differences of the SC within the SHR, with focus on visual and auditory information processing. Furthermore, this thesis investigated whether ADHD treatments, such as d-amphetamine and fluoxetine, affect SC responses, acting to normalise any physiological strain differences observed.

The SHR did show an increased response to visual stimulation, in the form of a light flash, both behaviourally and physiologically. In an SC-dependent behavioural task the SHR did not habituate to a non-novel, non-salient light stimulus in the same way as the two control strains, instead persistently responding to the stimulus throughout the 10 trials. From a physiological standpoint, there was no difference in likelihood of response at the different light intensities for local field potential responses, but the SHR was significantly more likely to have a multiunit activity response at the lower light intensities (4-12 Mcd) than the two control strains. The SHR also responded to the increasing light intensities differently, showing heightened multiunit activity in response to the 12 Mcd and 16 Mcd intensity, but no differences to the control strains for the final intensity, suggesting a ceiling effect. The SHR showed no significant differences in onset latency for local field potential data but did

have significantly longer onset latency than the WKY for the multiunit activity. As these key findings only occurred within the multiunit activity (outputs) and not the local field potential activity (inputs) this suggests that it is the internal collicular processing that produces the heightened responses in the SHR and not processes upstream in the retina. It was suggested that the physiological differences found could underlie the behavioural differences in the SC-dependent task. However, neither behaviour nor physiological measures could be related to gross morphological differences in collicular volume or cell counts, where there were no strain differences. In the pre-amphetamine baseline analysis, an increased visual multiunit response amplitude was seen in the SHR, yet no difference was seen in visual multiunit response onset latency, this may be due to the inclusion of the HL, a pigmented strain, or due to a reduced number of animals in this study, in *in vivo* electrophysiological studies a large intra-strain variability to the results in inevitable.

In terms of auditory processing, all animals responded and habituated towards the stimulus similarly. However, an opposite effect occurred to that seen in visual processing for the responsiveness to the stimulus in the physiological data. It is worth noting that the auditory response latencies found in this study were considerably shorter (3-8ms) than visual response latencies recorded in the superficial layers (40-70ms). There are different connections for auditory and visual inputs into the SC so a difference in latencies between different modalities within the SC is understandable. Furthermore, similar auditory collilcular response latencies have been seen in a variety of electrophysiological studies (Yeomans et al., 2006; Hungsun et al., 1984; King and Palmer, 1983). Although there was no difference in likelihood of a response to the different auditory stimulus intensities for local field potential responses, as was found for visual processing, the WKY was significantly more likely to have a multiunit activity response at the lowest intensity (55 db SPL) than the SHR and WIS, indicating that the SHR shows decreased responsiveness to auditory stimuli, at least with reference to the WKY. Beyond this responsiveness, findings were

broadly similar to the vision processing with the SHR having significantly longer onset latency for multiunit activity responses in comparison to the WKY, and also the WIS. This was also found for the local field potential data but only in comparison to the WKY. The SHR had significantly lower local field potential response amplitudes than the WKY and a trend towards significantly lower response than the WIS. However, these results were not significant in the multiunit activity (output) data. This suggests that the SHR has a reduced input into the deeper layers, potentially due to dysfunctional auditory processing upstream of the SC, for example, in the inferior colliculus (IC). It is arguable that the SHR has a hypo-functional IC-SC pathway, and the WKY has a hyper-functional IC-SC pathway, leading to strain differences found between these two inbred strains, yet no differences between either and the outbred strain, the WIS. As with the response differences in the superficial layers, the differences in the deeper layers cannot be attributed to any gross morphological differences such as volumes, neuron or glia cell counts, and therefore must be due to either differences in receptor densities, or differences in the number of different types of neuronal cells, i.e. a greater number of excitatory inputs compared to inhibitory ones. In the pre-amphetamine baseline analysis, a decreased auditory LFP and multiunit activity amplitude was seen in the SHR, yet no difference was seen in auditory multiunit activity onset latency, this may be due to the inclusion of the HL, a pigmented strain, or due to a reduced number of animals and large intra-strain variability in this study.

In order to investigate the effects of amphetamine in a way that could be compared to previous work with this drug (e.g. Gowan et al., 2008), the HL strain was also included in this study. Other than an increase in local field potential visual response amplitude at the lowest dose (0.5 mg/kg), amphetamine had a suppressive effect on LFP and multiunit activity visual responses, which was most pronounced at the higher doses (4-8 kg/Kg). This was true for all strains. Despite all strains reacting to the drug similarly in local field potential analysis, the HL did seem to be most responsive to the cumulative doses of

amphetamine in the multiunit activity responses in comparison to the albino strains with a greater suppressive effect at the higher doses (4- 8 mg/Kg). The fact that these differences only occurred in the multiunit activity data and not the local field potential responses suggests there are strain difference between the HL and albino strains in the effects of amphetamine within the SC superficial layers. Amphetamine had little effect on deeper layer auditory responses, only causing a suppressive effect on local field potential response amplitude as the dose increased (1-8 mg/Kg) and no effects on multiunit activity. All strains showed similar reactions.

Fluoxetine caused a suppressive effect on both local field potential and multiunit activity visual responses (2.5-10 mg/Kg). By contrast, it enhanced local field potential auditory responses, causing a significant increase in response amplitude, but suppressed multiunit activity auditory responses, causing a significant decrease in response amplitude. All the strains reacted similarly to the drug for both visual and auditory SC responses. Superficial and deeper layer 5-HT_{1B} and 5-HT_{1A} receptor densities were also examined within the three albino strains and found no significant differences.

7.2. THEORETICAL IMPLICATIONS

7.2.1. DYSFUNCTION IN THE COLLICULAR PROCESSING OF STIMULI

In the SC-dependent behavioural task, the SHR was found to have impairment in the ability to habituate to non-novel, non-salient visual stimuli. This lack of habituation suggests that the SHR has a heightened perception of visual saliency in comparison to the two control strains. This behavioural effect may arise as a result of dysfunctions in the collicular processing of visual saliency seen in these animals in Chapter 3. As there was no significant increase in the local field potential visual response in the SHR compared to the controls, it can be speculated that there is dysfunctional processing of visual information within the SC,

producing a significant increase in the multiunit activity in the SHR. According to Overton (2008) an overactive SC could issue stronger 'bids' for motor expression, to be the basal ganglia (Chevalier & Deniau, 1990) and therefore increase the likelihood of saccade generation and orientation towards a stimulus. By increasing the response, or 'bid' for weak stimuli, the SHR SC may bias the system so that distractions arise to non-salient stimuli. This would therefore cause an increase in overall distractibility and a deficit in sustained attention, as seen in people with ADHD. The finding that the SHR are physiologically more responsive to visual stimuli could underlie the effects seen in the present behavioural task but also other tasks conducted previously in both animals and those with ADHD (See Section 1.1.1 and 1.3.2.1).

As previously mentioned in Section 1.1.6, the key unifying theories of ADHD have focused a dysregulation of dopamine, despite the recognised limitations of only focussing on this neurotransmitter. The electrophysiological findings in the current study suggest that the dysregulation of dopamine may be a secondary effect of a dysfunction in the initial processing of salient stimuli within the SC affecting target selection based on saliency (Shen et al., 2011). A dysfunction within the colliculus could still elicit all the features described in the behavioural inhibition theory (see Section 1.1.6) put forward by Barkley (1997) as well as the developmental dynamic theory (see Section 1.1.6) put forward by Sagvolden (2005). As the SC has direct connections to midbrain dopaminergic neurons (Comoli et al., 2003; McHaffie et al., 2006), and has the capacity to activate and modulate their phasic activity (Dommett et al., 2005; Coizet et al., 2006), an increase in SC response would produce secondary effects in the phasic release of dopamine. A collicular dysfunction would also imply that the dysregulation in dopamine would be a secondary effect to the dysregulation of noradrenalin and serotonin within the SC, thus allowing for the importance of these other transmitters in ADHD pathophysiology.

The IC-SC pathway plays a crucial role in the generation of aversive and/or defensive motor commands. Morphologically, inferior collicular inputs are densest within the caudo-medial quadrant of the SC (Redgrave et al., 1987). This quadrant mediates eye and head orientation to the upper visual field (Tiao & Blakemore, 1976) and the organisation of behavioural escape responses in rodents crucially depends on this pathway (Dean et al., 1989). Stimulation of the tectopontine bundle, an uncrossed descending pathway arising from the SC produces avoidance behaviour in rats (Sahibzada et al., 1986). Inputs from the IC are densest in this SC region (Redgrave et al., 1987). Electrical or chemical stimulation of the IC induces fear-like reactions such as freezing, fight, or wild running (Cardoso et al., 1994; Pandossio and Brandão,1999). As the IC is a key source of acoustic information within the SC (Zwiers et al., 2004) and is crucial for SC-mediated aversive behaviour in rodents (Cohen and Castro-Alamancos, 2010), the findings that the WKY displayed more aversive and defensive behaviour during the auditory stimulus presentation in the behavioural task could suggest a disruption in this pathway within this strain. Physiologically, the WKY strain was significantly more likely to produce a multiunit activity response at the first intensity, and the SHR was significantly less likely to produce an local field potential response at the first intensity in comparison to the WKY. These findings, and as the SHR had significantly lower local field potential amplitudes than the WKY and a trend towards significant than the WIS, suggest that the SHR had a lower responsiveness to auditory stimuli in the SC than the WKY, and potentially the WIS. The differences in local field potential (input) responses indicate these strain differences are found prior to SC processing, such as a disruption or hypo-response to auditory stimulation through this IC-SC pathway in the SHR, while arguably a potential hyper-responsive IC- SC pathway in the WKY. It is worth noting here that the WKY is not a 'normal' control, and hence why the WIS was also used throughout experiments. Further evidence of this is that the WKY have been shown to have an enhanced startle response in comparison to Sprague-Dawley rats (McAuley et al., 2009), while the SHR has been found to have significantly lower startle

amplitude that the WKY, and Sprague-Dawley rats, with no difference in startle habituation (Van den Buuse et al., 2004). As the WKY strain was significantly more likely to produce a multiunit activity response at the first intensity, it suggests that this difference in local field potential (input) response has not been corrected within the SC processing, and will lead to a greater hypervigilance to low intensity auditory stimuli.

The findings of the SHR having significantly lower response auditory amplitude in comparison to the WKY in the present study, and a low startle response in Van den Buuse et al. (2004) are in line with earlier research on behavioural reactivity in these animals (Sutterer et al., 1988). As mentioned in Section 1.3.2.2. the SHR develops hypertension with age, and it has been shown that hypertension is an important pathophysiological risk factor in age-related hearing loss (Rarey et al., 1996). A deterioration in high-frequency (12- 24 kHz) hearing sensitivity occurs in aged (18 months) hypertensive rats (Borg et al., 1982). However, behaviourally there were no differences in auditory stimuli responding and habituation between the SHR and WIS strain. Similarly, as there was no responsiveness difference at the lower tone intensity between the SHR and WIS, it would suggest that this is not the case with the animals in this study. The animals used in this study were not as old as in Borg et al. (1982) study, and a lower (8 kHz) frequency was assessed. The lower amplitude response was only seen in the local field potential data and not the multiunit activity, suggesting that there is a lower auditory input in this SHR due to dysfunction in an area upstream. However, a hyper-responsive SC in the SHR compensates for this reduced input and therefore no significant differences in multiunit activity response outputs or behaviour were seen: responses and behaviours are normalised.

7.2.2. DELAYED DEVELOPMENT OF THE SUPERIOR COLLICULUS IN THE SHR

In the present study, the SHR had significantly greater onset latencies for both visual and auditory stimuli. This is in line with results from occulomotor paradigms, which are highly dependent on the SC, in which children with ADHD have also been found to have significantly longer saccade latencies in visually guided saccades (VGS, Mahone et al., 2009; Goto et al., 2010), memory guided saccades (MGS, Goto et al., 2010), prosaccades (Klein et al., 2003; Munoz et al., 2003), and antisaccades (Munoz et al., 2003; Feifel et al., 2004; Karatekin, 2006; Karatekin et al., 2010). Interestingly Wallace et al. (1996) found similar findings in the newborn monkey, where SC neurons had increased response latencies and approximately half the incidence of multisensory neurons when compared to adulthood. In other areas of the brain, this speeding of responses latencies occurs with development. For example, Sonntag et al. (2009) found a gradual shortening in response latencies up to P18 in mice when studying the development of sound-evoked discharge activity in the medial nucleus of the trapezoid body. This finding could suggest that the SHR has an incorrect development of this system, and may lead to the greater onset latencies seen in these animals: an interesting finding given ADHD is a developmental disorder. Similarly, children who received a cochlear implant, showed a significant decrease in minimum latency in brainstem auditory-evoked potentials within the first year of implant use, underlying mechanisms to produce this plasticity were likely due to improvement in synaptic efficacy and possible increased myelination (Gordon et al., 2003). It has been found that a delay or decrease in myelination has been seen in individuals with ADHD, and has been the suggested cause of abnormalities seen in the later maturing frontolimbic pathway in children with ADHD (Nagel et al., 2011). ADHD is a developmental disorder, with childhood onset (see Section 1.1), therefore a developmental delay of the SC leading to deficient collicular processing could link the longer onset latencies in the SC of the SHR and the saccade latencies seen in children with ADHD.

7.2.3. THE SC AS THE LOCUS OF ACTION OF ADHD MEDICATION

The role of the SC in saccade generation and complex tasks involving attention (see Section 1.2.5) makes it a potential site for the action of therapeutic drugs. Indeed, increased saccade latencies found in unmedicated children with ADHD can be normalised by treatment with methylphenidate (Mostofsky et al., 2001). The SHR did have significantly greater visual local field potential onset latency than the HL and a trend towards significantly greater onset latency than the WKY. This strain difference was not seen in the multiunit activity data, yet d-amphetamine did not normalise the responses seen in the SHR to comparable control levels.

The results of the present study suggest that d-amphetamine may (at least in part) act at the SC to moderate distractibility and improve sustained attention in these animals and could shed light on similar effects in individuals in ADHD, a clear association of this is that ADHD may be associated with collicular dysfunction. As it is well documented that amphetamine causes a decrease in distractibility in healthy subjects as well as 'healthy' rats, it is understandable that amphetamine will cause similar effects within the SC in the control strains and SHR used in this present study. Irrespective of this, amphetamine did reduce the increased responsiveness in the SHR seen in Chapter 3 indicating some normalisation of function. Amphetamine's mechanism of action in these animals and ADHD suffers could be to normalise these strain differences in the SC visual responses and thus normalise behavioural differences. Similar to this study, Gowan et al. (2008) found the administration of d-amphetamine produced dose-dependent depression of the amplitude and duration of responses to whole field light flash stimuli in the superficial layers of the SC. At the highest doses of D-amphetamine administered, visual responses were entirely suppressed. However, in the cat, D-amphetamine augmented responses in the superficial layers of the SC when a stimulus was displayed within the excitatory centre of the cells' receptive fields only (Grasse et al., 1993). It is possible that d-amphetamine amplifies the signal-to-noise

ratio as it suppresses responses to stimuli which give relatively minimal levels of SC activation, as in the sub-optimal whole field light stimuli (Gowan et al., 2008), and augments responses to stimuli which give relatively high levels of activation, such as stimuli limited to the excitatory centre (Grasse et al., 1993).

It was found by Dommett et al. (2009) that d-amphetamine supressed weaker collicular responses, while retaining stronger responses through pre-synaptic serotonin receptors. This work was done *in vitro* on brain slices, suggesting d-amphetamine does work locally within the SC. Isa et al. (1998) in similar slice work clarified the existence of a glutamatergic excitatory pathway from the optic tract to SGI neurons in the intermediate layers via the SGS in the superficial layers. Interestingly application of bicuculline, a $GABA_A$ receptor antagonist significantly enhanced the excitatory postsynaptic potentials recorded in the SGI neurons induced by stimulation of the optic tract or SGS, a finding reproduced by Dommett et al. (2009) to show amphetamine effects on responses within these layers. It suggests that there is a strong suppressive effect of GABA acting on this system. In light of this, these in vitro findings may suggest that a key requirement to the role of amphetamine acting within the SC is disinhibition. Such disinhibition is present in awake, but not anaesthetised animal, as indicated by intermediate visual responses in awake animals in the absence of artificial disibihibition (Brecht et al., 2001). This could explain the lack of clear effects in the current study which did use an anaesthetised preparation.

The implication of amphetamine's effects from a behavioural standpoint, is that it has been hypothesised that the SC could 'bid' for motor expression, thus, heightened activity can be thought as placing a stronger "bid" into the central selection device thought to be the basal ganglia (Chevalier & Deniau, 1990), therefore increasing the likelihood of saccade generation. The superficial layers of the SC have a direct ascending projection to the thalamus and then forward to the neostriatum (McHaffie et al., 2005), and also a

connections with the deep layers of the SC (Lee et al., 1997), which also project to the thalamus (McHaffie et al., 2005). By efficiently decreasing the response, or 'bid' to weak stimuli, psychostimulants have the ability to bias the system so that distractions (a motor output and hence a saccade) only arise to predominantly salient stimuli. By enhancing SC responses, the likelihood of a saccade could be improved. Conversely, as is the case with d-amphetamine, by depressing responses in the SC, the prospect of a saccade would be lowered. This would therefore cause a reduction in overall distractibility and a correlative enhancement in sustained attention, as seen in normal people, ADHD sufferers, as well as rats and more specifically the SHR following psychostimulant administration.

Boulenguez et al. (1995) hypothesized that 5-HT, via 5-HT_{1B-1D} receptors, may cause an increase of the visual distractibility threshold by modulating directly the transmission of the primary visual signal. Serotonin is the dominant monoamine affected by amphetamines in the superficial layers of the SC (see Section 5.4). Further supporting this theory, Dommett et al. (2009) found that therapeutically relevant doses of D–amphetamine and methylphenidate increased the signal-to-noise ratio in the SC by suppressing weak and preserving strong activations mediated by serotonin via a pre-synaptic mechanism. It has been shown that serotonin reduces the signal-to-noise ratio in the somatosensory cortex (Waterhouse et al., 1986) and thalamus (Funke & Eysel, 1993), yet not by altering the weak and strong signal relationship but by suppressing spontaneous background activity. The combined effect of 5-HT at both subtypes ($_{1A}$ and $_{1B}$) would bias SC visual activity toward information received from the corticotectal pathway. In light of this, it is further suggested (Mooney et al., 1996) that 5-HT gates retino-collicular input to the SuG via pre-synaptic receptors as a means of selectively enhancing the relative contribution of cortical input in SuG (at the expense of retino-collicular input) during periods of arousal. The behavioral effects of fluoxetine and amphetamine could be linked to a change in the signal-to-noise ratio effect mediated by serotonin in the SC by biasing the system towards salient stimuli

and consequently leading to a reduction in distractibility, and could underlie key processes involved in the adaption of the reactivity according to the state of arousal of the animal.

D-amphetamine has been shown to act locally within the superficial layers of the SC to affect visual responsiveness, however the lack of amphetamine effects on multiunit activity auditory responses could be due to the distinct differences in the distribution of neurotransmitters within the SC. Expansive serotonergic innervation, preferentially innervating the superficial layers is found in the SC (Parent et al., 1981; Weller et al., 1987), while restricted noradrenergic (Lindvall & Bjorklund, 1974; Weller et al., 1987) and dopaminergic input (Weller et al., 1987; Campbell et al., 1991) have been reported. Similarly, the concentration of endogenous noradrenalin was reportedly higher in superficial than in deep layers of the superior colliculus (Wichmann and Starke, 1988). This suggests that serotonin is the dominant monoamine affected by amphetamines in the superficial layers of the SC, yet little monoamine transmission occurs within the deeper layers.

As previously mentioned, all animals responded to fluoxetine (Chapter 6) in the same way. Similar findings were seen in Chapter 5 with amphetamine application. Amphetamine and fluoxetine caused a suppressive effect on visual responses. Amphetamine caused a significant decrease in local field potential and multiunit activity visual responses by the final dose for all parameters (increasing onset latency, decreasing amplitude and duration). It has already been noted that higher doses of amphetamine work on serotonin and supress visual responses. Similar to the effect of amphetamine, as the effects of fluoxetine reduced the increased responsiveness in the SHR seen in Chapter 3 potentially by increasing the distractibility threshold to stimulus, and therefore effectively altering the signal-to-noise ratio, fluoxetine's mechanism of action in these animals and ADHD suffers could be to normalise these strain differences in the SC visual responses and thus normalise

behavioural differences. However, the drug effects seen in the present experiments presented in this thesis do not support amphetamine or fluoxetine fully normalising the strain differences seen in the collicular responses, which could suggest a lack of predictive validity in the SHR, in line with some other studies (Warton et al., 2009; Van den Bergh et al., 2006).

7.3. VALIDITY OF THE SHR

Within this study, the SHR has been shown to have face validity because, as expected, the SHR was consistently distracted by visual stimulus and did not readily habituate to it in comparison to the control strains. Yet, the SHR lacked face validity in the auditory behavioural study as all animals responded and habituated towards the stimulus similarly. However, it should be noted that the auditory stimulus may have been too intense to find habituation within the number of presentations tested.

Construct validity of the SHR is supported in this study by the increased responsiveness to lower light levels physiologically. The SHR was significantly more likely to respond, and had greater response amplitude to visual stimuli (see Section 7.2.1). Both of these finding support validity because they show that alterations to a structure that impacts on attentional processing have occurred. The greater onset latency found in the SHR in this study further supports the construct validity of the SHR as the strain could have incorrect development of this system, leading to the greater onset latencies seen in these animals, an interesting finding given ADHD is a developmental disorder (see Section 7.2.2). The smaller whole brain volume of the SHR also supports the construct validity of these animals (see Chapter 3). By contrast, the lack of significant differences in serotonin receptors between the SHR and the two control strains challenges the construct validity of the SHR as

serotonin is a key neurotransmitter within the SC and is implicated in ADHD pathophysiology.

As described above, all animals responded to fluoxetine and amphetamine in a similar way, both causing a suppressive effect on visual response. However, neither drug fully normalised the strain differences seen in the collicular responses. This can be seen as a challenge to the predictive validity of the model, although it should be borne in mind that there is a high rate of non-responding to ADHD medication (Newcorn et al., 2008) and therefore this challenge is limited.

7.4. METHODOLOGICAL ISSUES OF THE CURRENT EXPERIMENTS

All the physiological studies completed here were conducted in the anesthetised rat and therefore results may differ from those obtained in an awake animal. Any differences may be contributed to, in part, by the effects of the specific anaesthetic employed in the current study because urethane has been found, albeit in a study focussing on nociceptive neurons, to be able to both enhance and suppress neural activity in different populations of collicular neurons (Wang et al., 2000). However, the ability of different anaesthetics to modulate collicular responses is not limited to nociceptive processing, as Binns and Salt (1995) found effects of anaesthetics on visual responses in the cat colliculus. Furthermore, these anaesthetic induced effects are not limited to the SC and can influence other subcortical structures. For example, basal activity of nigrostriatal dopamine-containing neurons in the rat is reduced under urethane anaesthetic as compared with unanaesthetised paralysed controls (Kelland et al., 1990). However, although the current findings may not necessarily be generalised to the awake animal, given the main aim of this thesis was to find strain differences between the SHR and the two control strains, the WIS and WKY, and all animals

were kept at the same anaesthetic depth, the results presented can still provide useful information about the potential neural basis of ADHD behaviours.

There are also effects of urethane outside of the brain that could have impacted on the present study, in particular, the ability of urethane to lower blood pressure (Hillebran et al., 1971). This may be particularly pertinent in the work conducted here because the SHR is also used as an animal model of hypertension. Blood pressure effects of urethane are dependent on the route of administration, the sex of the animal and the time since administration. An intraperitoneal injection of 1.2 mg/Kg urethane to male WIS and Spague-Dawley rats caused no change in mean atrial blood pressure and heart rate (Carruha et al., 1987). By contrast, the same route and dose given to female WIS rats caused a decrease in mean blood pressure to 95 mmHg, compared to 125 mmHg in unanaesthetised animals, which persisted for at least 1 hour after injection (Hillebran et al., 1971). Given the sex effects, it is likely that any reduction in blood pressure would be reduced in the present study because only male rats were used. Furthermore, following surgical preparation and the period of light adaption prior to recordings being made, animals in the present study would have been under anaesthetic for over an hour, Finally, the fall in blood pressure after intra-peritoneal injection of 25% urethane at 1 mg/Kg can reportedly be reduced by slow injection (Van Der Meer et al., 1975), and the animals in the present study were given an initial light dose, and gradually topped up until the removal of the reflexes occurred, further mitigating the effects of urethane on blood pressure. In summary, the exact experimental procedures used would indicate that changes in blood pressure induced by urethane would not have had a significant impact on the results presented.

In addition to the potential impact of anaesthetics, the route of administration and doses of the two drugs used can be considered limitations of the current study. Considering first the

route of administration, both drugs were given intravenously and therefore it is difficult to establish whether the drug effects on visual and auditory responses were purely due to drug actions within the SC, and not due to drug effects altering other structures and systems that could in turn alter collicular processing. For example, dopamine is known to be a key neurotransmitter in the retina of all vertebrates and therefore, amphetamine at least, could exert some effects at the retina that would have the potential to alter visual responses (Piccolino et al., 1987). In addition, serotonin is thought to play a key role in the development of the retinotectal pathway (Bastos et al., 1999), providing a possible upstream locus of action for fluoxetine to influence visual responses. Similarly, fluoxetine has been shown to depress the activity of the IC (Jang et al., 2009), a key structure upstream of the SC. However, as the response data was split into local field potentials and multiunit activity it can be argued that any differences occurring in the local field potential data may represent changes prior to collicular processing, while differences in the multiunit activity suggest changes to processing within the SC itself. In support of the effects on visual responses being due to changes to collicular processing, Gowan et al. (2008) found similar suppressive effects on visual responses to the current experiment following intra-collicular administration of amphetamine.

Agonists of 5-HT_{1B} and 5-HT_{1D} receptors, known as "triptans" used for migraine treatment, bind to receptors localised on cranial vessels leading to vasoconstriction and a decreased release of neuropeptides involved in "sterile inflammation" (Ahn and Basbaum, 2005). This could suggest the differences seen in fluoxetine, a serotonin reuptake inhibitor are due to changes in blood pressure, notably already a problem in the SHR, yet as there were no strain differences in the effects of the drug on responses, with all the animals responding similarly to the drug, this is unlikely to be the case. Any changes in response due to fluoxetine, will notably be more pronounced in the SHR if the strain differences seen in the SHR are due to its hypertension. Also, if the lowering of blood pressure did occur, as the

SHR is known to be hypertensive, the lowering of blood pressure should not be a problem, as if anything it may lower this animal's blood pressure to control comparable values, minimising this variable.

As well as the difficulty isolating the locus of action when a drug is administered intravenously, it is also not a therapeutic route of administration for ADHD medications, which are given orally to individuals with the condition. This means that there are likely to be key differences in the pharmacokinetics of the drugs in the present experimental paradigm when compared to use in people (Kuczenski and Segal, 2005). For example, the oral route normally results in lower peak drug concentrations and a greater rate of drug accumulation in comparison to injections. Furthermore, it is difficult to accurately translate therapeutic doses given orally in humans to those given by injection in rats. One way is to consider the blood plasma levels required to achieve a therapeutic effect in people with ADHD and attempt to match this in the experimental animal. However, whilst the blood plasma levels of therapeutic doses are known for amphetamine to be around 120 ng/ml (McGough et al., 2003; Ricaurte et al., 2005), there is presently a lack of understanding as to how these translate to drug levels within the brain and if this varies with species. Furthermore, there is no such data for the use of fluoxetine in people with ADHD. An alternative approach is to examine the concentration needed to achieve appropriate occupancy of the relative transporter within the brain itself. Schiffer et al. (2006) used PET to show that an amphetamine dose of 0.5 mg/Kg, i.v. produced DAT occupancy levels in the primate brain equivalent to those achieved by the therapeutic human doses. Meyer et al. (2004) found that 80% occupancy of SERT (serotonin transporter) is important for therapeutic effect in humans, whilst Ginovart et al. (2003) reported 90% SERT occupancy using PET in the striatum, midbrain, and thalamus of cats 30 min after 1 mg/Kg, i.v. fluoxetine administration. Li et al. (2010) also found similar occupancy levels in the rat following 5 mg/Kg s.c. (subcutaneous injection), which would result in bioavailability

corresponding to lower i.v. doses. Combined these results, suggests that the lowest two doses of fluoxetine used in the present study are arguably comparable to therapeutically relevant doses in humans but that our higher doses may have exceeded average therapeutic levels. That said, studies have been shown to use concentrations comparable to this study in rats to produce a behavioural effect to amphetamine (Gowan et al., 2008, Clements et al., 2014) and fluoxetine, such as a reduction in exploratory location and attention (Dringenberg et al., 2003; LaRoche and Morgan, 2007).

It is important to note that even though the anaesthetic depth, pharmokinetics and route of administration of drugs used do make the comparison of drug doses between studies confounding. It can be suggested that the lowest doses of amphetamine used in this study may be in therapeutic range, and have been found to improve behaviours in rats. Bizarro et al. (2004)and Sagvolden and Xu (2008) found improvement in impulsivity and attention in rats in visual discrimination tasks following 0.1-1 mg/Kg dose and 0.5-2 mg/Kg of amphetamine respectively. Yet, the higher doses used in this study have been shown to cause increased locomotor and stereotypic behaviour in rats, and may be out of therapeutic range. As Porrino et al. (1984) found 1.0 mg/Kg i.v. dose of amphetamine produced increased locomotion and stereotypic sniffing, while 5.0 mg/Kg produced stereotypic gnawing and licking in the animals studied. Conversely, in a double-blind study of 45 hyperactive boys, Borcherding et al. (1990) found that 34 children exhibited stereotyped orofacial movements, compulsive hand motions, repetitive eye blinking or head jerking following amphetamine treatment.

The ideal SHR animal model of ADHD has been noted to be in juvenile phase of development, prior to the onset of hypertension. Juvenile SHRs show all ADHD-like behaviours, such as attentional deficits, impulsivity as well as hyperactivity. The hyperactivity symptom is specifically only seen in the juvenile (4-6 week old) stage of their development, prior to the development of hypertension (Sagvolden et al., 2005). It is worth

noting that behavioural testing for attention and impulsivity take a longer duration to train than a test for hyperactivity, so it is questionable as to whether these animals are only 4-6weeks old during testing. Despite this, the use of adult rats may affect the generalisability of the results seen in this work when comparing to juvenile SHRs and juvenile human suffers. Similarly, differences in ADHD-like symptoms have been found to be dependent on the sex of animal used, for example Berger and Sagvolden (1998) found inattentive deficits to be more pronounced in the female SHR when compared to male SHR and the WKY control. Despite this suggesting face validity as the predominantly inattentive presentation of ADHD is most prevalent in human female suffers (Taylor et al., 1998), it can also be argued that the use of male SHRs in these studies may suggest these findings will similarly lack generalisability for female suffers and female SHRs.

Finally, all studies were carried out in the rat and it is not clear if these findings would generalise to other species, including non-human primates. It is likely that some aspects of the current work on visual and auditory processing are applicable to the monkey, because the responses of SC neurons to these stimuli are similar (McPeek & Keller, 2004; Lovejoy & Krauzlis, 2010). In addition, the SC focused on in the present research are subcortical systems that are highly conserved across species (Overton, 2008), suggesting that these findings are applicable to all species.

7.5. FUTURE DIRECTIONS

Further work on visual and auditory stimulus responses

Due to the strain differences in the responsiveness to lower stimuli intensities seen in Chapter 3 and 4, where the was an increased likelihood of the SHR to respond to lower levels of visual stimulation, and were less likely to respond at lower levels of auditory stimulation, an important unanswered question would be the effects of drug administration on SC responses at lower stimuli intensities. This experiment would also shed light on the

differences seen in Dommett et al. (2009), in light of a dysfunction in the signal noise ratio in the SC.

Strain differences in responses upstream and downstream of the superior colliculus

A future study looking in to the effects of auditory stimuli on the inferior colliculus and other auditory structures upstream of the SC in these animals may shed light on the proposed dysfunction in the local field potential auditory SC responses seen in this thesis. The greater role of ascending inputs on auditory stimuli may also explain differences between the auditory and visual behaviour task as this defensive IC-SC pathway is a more crucial component of behavioural outcomes to auditory stimuli, than ascending pathways for visual processing.

As previously mentioned, the electrophysiological findings in the current study suggest that the dysregulation of dopamine may be a secondary effect of a dysfunction in the initial processing of salient stimuli within the SC affecting target selection based on saliency (Shen et al., 2011), and therefore, causing impairments in behavioural inhibition to non-salient stimuli in these animals, and potentially in individuals with ADHD too. A future experiment looking at the SC direct connections to midbrain dopaminergic neurons (Comoli et al., 2003; McHaffie et al., 2006), and if the capacity to activate and modulate their phasic activity (Dommett et al., 2005; Coizet et al., 2006) is different and more exaggerated within the SHR in comparison to the two control strains.

Further work on drug application on superior colliculus responses

Gowan et al. (2008) following intra-collicular administration of amphetamine found similar suppressive effects on visual responses as within their paper (Gowan et al., 2008) and this thesis. These experiments were done on the HL strain, therefore it would be interesting to see the effects of amphetamine, as well as fluoxetine following intra-collicular

administration on the SHR. This would also further establish whether the SC is the locus of ADHD treatments such as amphetamine and fluoxetine. It would also be interesting to see amphetamine and fluoxetine effects on a range of stimulus intensities to shed any light on a dysfunction in the signal-to-noise ratio in the SC of the SHR.

Due to the strain differences seen in the SC-dependent behavioural task, as well as the physiological response data, it would be interesting to see what effects amphetamine and fluoxetine have on the SHR within this task, establishing whether the lack of habituation seen in these animals towards visual stimuli would be normalised, and comparable to the control strains following drug treatment. Similarly to the experiment above, it would also be interesting to see the effects of intracolliclur administration of amphetamine and fluoxetine.

Further work on freely moving animals

The key physiological findings of this thesis, as previously mentioned are on an anaesthetised animal, this means results cannot be generalised to an awake animal. Given the main aim of this thesis was to find collicular response strain differences between the SHR and the two control strains these findings do still provide useful information about the potential neural basis of ADHD-like behaviours in this strain. Yet as results cannot be generalised to the awake animal future experiments into collicular response differences between the SHR and the control strains in freely moving animals through electrode implantation would further shed light on strain differences in this area. It would also be a highly useful experiment in looking at ADHD medication in on the animal's behaviour in an SC dependent task concurrent with SC response changes to really shed light on link between the drugs physiological and behavioural effects.

Further work on morphological differences within the superior colliculus

As there were physiological and behavioural differences seen within the SC of the SHR compared to control, yet there were no morphological differences i.e. cell counts, 5-HT_{1B} receptor density. A future experiment to shed light on the cause of these behavioural differences would also be crucial, such as differences in glutamate receptor densities, or differences in the number of different types of neuronal cells, i.e. a greater number of excitatory inputs compared to inhibitory ones for example. Also, a low number of subjects were used within the immunohistochemistry and morphological experiments, which potentially could of given these experiments low statistical power. A study with low statistical power has a reduced chance of detecting a true significant effect, and also reduces the likelihood that a statistically significant result reflects a true effect (Button et al., 2013). The consequences of this include overestimates of effect size and low reproducibility of results. An increased number of subjects may pull apart any strain differences, such as an increased 5-HT_{1B} receptor density in the SHR in Chapter 6, but due to the high amount of variance, this was not significant.

7.6. FINAL CONCLUSIONS

Understanding the etiology of the ADHD-like behaviours in the SHR is important in improving our understanding of the etiology of ADHD itself. Furthermore, improved understanding could also result in the discovery of new treatments for ADHD, devoid of actions on the dopamine system, and therefore potentially lacking abuse liability. This thesis has presented work that demonstrates that the SHR responds to visual stimuli in a different way to the two control strains, with these differences likely to be mediated by mechanisms within the SC which result in increased saliency of sensory stimuli. This in in line with the two unifying theories on ADHD (Barkey, 1997; Sagvolden, 2005), yet suggests the dysregulation is upstream in the SC rather than primarily at dopaminergic neurons.

Similarly, the findings of greater onset latency in these animals are in line with ADHD and the ADHD-like behaviours seen in the SHR are due to the condition being a developmental disorder. ADHD treatments such as amphetamine and fluoxetine may have a mechanism of action within the SC, and therefore normalise the exaggerated response, yet the results from the current study are inconclusive. Future work needs to investigate the dysfunction in the SC of the SHR for a range of stimuli intensities, to determine whether there is a change to the signal-to-noise ratio in these animals as has been previously suggested for ADHD, and also, whether these drug's therapeutic effects are on the signal-to-noise ratio mechanism within the SC.

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